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Volume LXXXI Number 2

The BOTANICAL GAZETTE

FOUNDED BY JOHN M. COULTER

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THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, London
THE MARUZEN-KABUSHIKI-KAISHA, Tokyo, Osaka, Kyoto, Fukuoka, Sendai
THE COMMERCIAL PRESS, LIMITED, Shanghai

Volume LXXXI Number 2

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of members of the botanical staff of the University of Chicago

Issued April 13, 1926

The Botanical Gazette is published in eight issues annually (omitting January and February, and July and August) by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is $1.25. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, El Salvador, Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Peru, Uruguay, Paraguay, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, and Spain. ¶Postage is charged extra as follows: For Canada and Newfoundland, 30 cents on annual subscriptions (total $9.30); on single copies 4 cents (total $1.29); for all other countries in the Postal Union, 60 cents on annual subscriptions (total $9.60), on single copies 8 cents (total $1.33). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

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Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

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Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

PRINTED IN THE U.S.A

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For March 1926 *and April 1926*

Individualism or Socialism?

J. Maurice Clark, who gave us in *The Economics of Overhead Costs*, a pioneer work on the theme of unused capacity, makes in his new book, *The Social Control of Business*, an experiment in the interpretation of one of the most all-embracing aspects of economic life. He has analyzed the question of adjusting conflicting interests and claims of rights in modern economic life, and since individualism and socialism are the outstanding alternative schemes for accomplishing this end, he has much to say about both. Other systems of control involved are state socialism, revolutionary communism, syndicalism, and anarchism.

Mr. Clark's conclusion is that industry is a matter of public concern and that "the stake which the public has in its processes is not adequately protected by the safeguards which individualism offers." But he goes on to say, "Society does not know what it wants or cannot devise means that will secure it. Hence, the lesser evil is to let individualism hold the field. Such a situation may be regrettable but it is an evil from which we suffer less, on the whole, than from the meddling and muddling that results when the government acts without knowledge."

In this book, as in no other reference on the subject, the entire field of social control is organized and clarified for the student. A college text for collegiate schools of business, it is designed for the survey course in social control, whether it introduces advanced courses in special problems or follows them, tieing together what would otherwise remain separate bodies of material.

Chief emphasis is placed upon the problems common to trusts, railroads, and public utilities arising out of fixed capital, untraced expenses, increasing returns, and resulting tendencies to monopoly. Complementary phases of control treated are common law, statute law, and codes of economic ethics.

Part II of the volume concentrates on a group of definite and tangible problems: price control, public utilities, and the question of public ownership—that is to say, a large and significant section of the entire field of economic life.

It is Mr. Clark's belief that industry has physical capacity and technical ability to produce far more than it does, but is kept short of its capacity by a failure of social co-ordination; and that the greatest field open to human invention is in making improvements in our system of social control. He has shown how the machinery of social control is actually working, and has pointed out the features of what he considers a desirable system of control.

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* * *

Evolution Up-to-Date

Recent years have witnessed important changes in various phases of evolutionary science. To take account of recent develop-

ments, Horatio Hackett Newman has completely revised his comprehensive treatment of evolutionary biology, *Evolution, Genetics, and Eugenics*. The new edition is entirely up-to-date, and is greatly improved from the pedagogical point of view. The discussion of mutations, eugenics, and other topics has been revised in the light of work done since the appearance of the earlier edition. Much new material has been added. A chapter on mutations has been written especially for the book by R. Ruggles Gates, and there is a new paper by H. F. Muller. The section on eugenics has been strengthened by the addition of a lucid chapter from Albert E. Wiggam's book *The Fruit of the Family Tree*. New matter appears in Professor Newman's connecting and introductory statements, and the chapter on "Linkage and Crossing-Over" has been re-written in a more elementary style for the beginner.

Personal attendance at the trial of J. T. Scopes for violation of the Tennessee anti-evolution law has enabled Professor Newman to describe it in this new edition in connection with his general interpretation of the present anti-evolution campaign in the United States.

The order of presentation of the material has been changed from one based on the degree of directness of the evidence to one governed by the logical succession of topics. This new order gives the text added clearness and teachability.

The reading materials contain certain technical terms that are often perplexing to the beginner. This is remedied in the revised edition by the addition of a full glossary which defines nearly all the biological terms used in the book.

In a single volume, a survey of the entire field of evolutionary science—this is what Mr. Newman has achieved in *Evolution, Genetics, and Eugenics*. With original material and connective and interpretative sections, the author weaves into a unified account a well-balanced selection of excerpts from such writers as Darwin, Wallace, Romanes, DeVries, Le Conte, Morgan, Castle, Lull, Guyer, Popenoe, Shull, Downing, and Thomson.

The revised edition is the most complete and up-to-date textbook available for survey courses in the subject, and is a most readable compendium for the general public. *EVOLUTION, GENETICS, AND EUGENICS*. By HORATIO HACKETT NEWMAN. (Revised Edition.) *$3.50, postpaid $3.70.*

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A Living Wage

In *Wages and the Family*, Paul H. Douglas points a way out of the *impasse* in which the living-wage principle finds itself, due to the fact that most of its adherents have used it to support the impracticable contention that men should be paid enough to maintain a "standard" family of five. He accomplishes this by championing a basic wage plus the payment of allowances for dependents.

That industry cannot be saddled with the maintenance of forty-five million fictitious wives and children, Dr. Douglas makes clear, and he argues that although industry should pay a living wage, it should not, and cannot hope to, pay all adult male workers enough to support a family of five.

He proposes three features which he believes essential to any proper wage system: the payment of an adequate basic wage, the payment of allowances for dependents, and the creation of an equalization fund to obviate discrimination against hiring those with dependents. With this latter feature there has been a great deal of experimentation in France and Germany. This book is particularly concerned with working out the details of an adequate and just system in the light of American conditions. *WAGES AND THE FAMILY*. By PAUL H. DOUGLAS. *$3.00, postpaid $3.15.*

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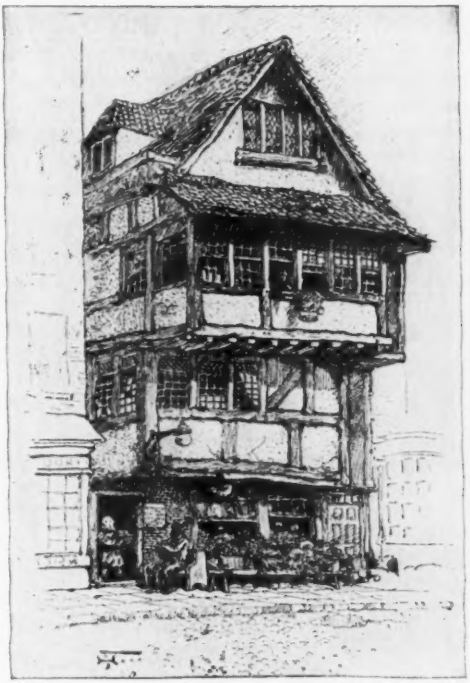

The Outlook (London) says of it "For use of students of English history on its social side the book is of highest possible value. . . . . It has values far transcending mere utility and we do not think we praise it too highly when we count it worthy to rank with the literature it has used so adroitly." *LONDON IN ENGLISH LITERATURE.* By PERCY HOLMES BOYNTON. *$2.50, postpaid $2.65.*

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VOLUME LXXXI NUMBER 2

THE
BOTANICAL GAZETTE

April 1926

STUDIES IN THE GENUS XANTHIUM

JENNIE L. SYMONS

(WITH PLATES IX–XI)

Introduction

The studies in the genus *Xanthium*, of which this is a preliminary account, were begun in the autumn of 1922. An attempt was made to obtain seeds from all parts of North America, thus to gain some idea of the distribution of species. It was hoped, also, that a study of the plants, from germination to maturity, would throw some new light on the taxonomy by revealing genetic relationships in the genus. An experimental garden has been devoted exclusively to *Xanthium* for two summers. In 1923, seeds from 22 different sources in North America were grown. In 1924, all the seeds planted had been collected in two small areas on opposite shores of the St. Lawrence River at Montreal. Small numbers of plants have been grown in the McGill University greenhouse during the winter months of both years. Observations have been made upon the main differences in habit, foliage, relative time of flowering, and other points not easily determined from herbarium material. Breeding experiments have been carried on, particularly on the St. Lawrence River forms. As a check upon the results thus obtained, some cytological studies and chromosome counts have been made. Incidentally, notes have been taken during both summers upon the occurrence of delayed germination in the garden.

In the beginning of the investigation, letters were sent to the Departments of Agriculture in Washington and in Ottawa, and eventu-

ally to all the Agricultural Experiment Stations in the United States and Canada. The response to these letters was very generous. Burrs were received from 22 different sources in North America, and from 4 different parts of Europe. When burrs were still attached to the plants in the field, trouble was taken to wrap fruits from single plants in separate packages, in accordance with my request.

Several collecting trips were made to the St. Lawrence River shores, at Point aux Trembles on the north side and at Longueuil on the south.

Plants were taken in the field, numbered, wrapped separately in newspaper packages, and brought to the laboratory. There they were stripped of their burrs, which were kept in glass jars until spring. The same collecting grounds were visited again in May, when the seedling plants had just unfolded their cotyledons; 107 of these small plants were taken up and successfully transplanted into the experimental garden. On the same collecting trips large masses of detached burrs were found mixed with rope waste and other material which had been washed on shore when the river was at its highest level. Numbers of these were gathered for planting in the garden.

Method

Plantings were made 2 feet apart. The rows were 2.5 feet apart as a rule. The location of each seed was marked by a wooden label on which the source of the seed and the date of planting were noted. Fruits which had been classified as belonging to the same species were planted in the same part of the garden. In the first season, fruits and seeds were planted from each lot received. In each case, half the burrs were planted whole and half were opened, the seeds then being planted separately. In the second season, fruits and seeds from 42 different plants were sown, as well as the detached burrs collected in the spring. The seedlings were planted first, 2.5 feet apart, in the center of the garden. In both seasons the planting was done during the first week in June.

When the buds first appeared, plants were selected for breeding experiments. Staminate and pistillate flowers being separate in *Xanthium*, pollination is easily controlled. Staminate heads were removed with forceps which were sterilized by dipping in absolute

alcohol. Several plants of each type grown were thus emasculated and bagged before the stigmas had appeared in the female blossoms. Others were bagged to insure purity of pollen for the controlled pollination. Between four and five hundred branches were thus protected. The bags were of the usual square-bottomed sort, of strong brown paper, in medium size. To facilitate pollination, the bottom of the bag was cut around three sides and made fast again with paper clips. The blossoms could thus be observed from time to time and treated when sufficiently developed. In transferring pollen from one plant to another, it was found most practicable to cut the male branch and carry it, still inclosed in its bag, to the branch to be pollinated. There, both bags were carefully opened and the pollen shaken in upon the exposed stigmas. Many crosses were tried, with all possible combinations of species. Some branches of each kind were left for self-pollination. In each species some branches which had been emasculated were left unpollinated to test for apogamy. In a few cases, pollen was shaken up with a little pure water and applied with the finger-tip, a method which also succeeded. After pollination, the clips were replaced and the bags left until late September. After a heavy rain, it was usually necessary to loosen the cord which tied the bags at the base of the branch to let out any water which had accumulated. A few branches being attacked by fungi in September, when the fruits were well developed, the bags were removed. Fruits were gathered in late October.

For the cytological work, staminate heads were collected when young from all species and varieties in the garden. The fixing fluid was taken into the field in small bottles which were labeled as the material was gathered. A modification of Bouin's fluid was used for fixation. It was made up according to the directions of ALLEN (1) as follows:

Picric acid (sat. aq. sol.)	75 cc.	
Formol	25 cc.	
Glacial acetic acid	5 cc.	
Chromic acid crystals	1.5 gm.	Dissolved in the fluid just before use
Urea crystals	2.0 gm.	

After 1–3 hours in the fixing fluid, the material was washed in 75 per cent alcohol until no yellow color remained. It was preserved in

70 per cent alcohol until imbedded in paraffin. The sections were stained with iron-alum haematoxylin. The best results were obtained when slides were left in the haematoxylin at least 24 hours.

For the fixation of material grown in the greenhouse Carnoy's fluid was used. This fixative was as satisfactory with *Xanthium* material as the more elaborate one just described. The smear method, as recently outlined by TAYLOR (22), was also tried with some success.

Investigation

1. TAXONOMY AND GENETICS

In the first season, as the plants grew, the variation in habit and foliage was greater than had been expected. With a few exceptions, the habit of plants from different sources differed enough to enable even a casual observer to distinguish readily the point in any row where plants from one locality adjoined those from another.

None of the southern forms blossomed in time to produce any fruits, although the plants were large and healthy. Plants from farther north grew less luxuriantly, but matured abundance of fruit. The burrs were similar to the ones which had been planted in each case, although in some instances it was difficult to identify them with certainty from the available classifications. Eventually, specimens of all these forms were taken to Washington for comparison with material in the National Herbarium. In many cases, conclusions already reached were confirmed, but some uncertainties remained. The greatest difficulty was with forms collected on the banks of the St. Lawrence River, near Montreal. MILLSPAUGH and SHERFF (11), in their first paper on *Xanthium*, state the condition of affairs as they found it as follows:

> In determining certain specimens of *Xanthium* in the Herbarium of the Field Museum, the writers have found the taxonomic status of this genus, as concerns its various species, to be very unsatisfactory at the present time. Much of the uncertainty in connection with several species arises from the difficulty encountered in the past in identifying the older specific names, names that in a number of cases, at least, were founded upon heterogeneous material. . . . . Another source of confusion in herbaria has been the erroneous identification of new and undescribed species with any one of the older, more commonly known species. Still further, we must note the well-known contempt with which common weeds such as *Xanthium* are so often regarded, a reason that explains the

surprisingly small number of herbarium specimens of any one species collected heretofore in a given region.

Later, in their monograph on North American species of *Xanthium*, in which they thoroughly review the taxonomic literature of the genus and greatly reduce the number of problems in bibliography, they point out the less easily solved difficulty as to species concept.

> The species concept in *Xanthium* . . . . must long remain a perplexing problem. In temperate regions the plants do not mature their fruits sufficiently for exact determination until after frost comes and the majority of collecting botanists have ceased their field work. This renders good material in herbaria scanty in quantity and inadequate in quality. Some of the species are known to exhibit most striking variations in fruiting characters, variations that with many botanists would be taken to represent varieties or subspecies. . . . . We have retained several of the less well-known species because we have felt that only after further field observations and breeding tests can satisfactory conclusions as to their true status be reached.

Some forms still defying classification, it seemed futile to describe them as new species until their purity was established. This certainty as to purity of species seemed also necessarily to precede any real conclusions about their distribution. It was therefore decided to devote the summer of 1924 to breeding experiments upon a limited number of forms. Those collected on the shores of the St. Lawrence River at Montreal were chosen. There were several reasons for this choice. First, abundance of material was available and field observations could be made at different seasons as a check upon experimental results. Second, some of the most interesting and baffling of the forms thus far observed had been collected in this locality. It seemed highly probable, owing to the action of the river as a distributing agent, that a greater number of species would be found in a small area here than in most places. If natural conditions permitted hybridization in the field, many hybrid forms would also be found.

LASCH (**6**), as early as 1856, finding in one locality three species of *Xanthium* and several forms which combined certain of their characteristics, described the latter as hybrids. BITTER (**2**), however, was the first to do actual breeding experiments with species of *Xanthium*. He reported, in 1908, that he had successfully crossed *X. macrocarpum* DC. and *X. italicum* Mor. with *X. strumarium* L. His cultural studies had led him to the conclusion that within the

section Euxanthium hybridization could take place freely, although crosses could not be made between any of these species and *X. spinosum* of the section Acanthoxanthium.

SHULL (17) found that the three main types of *Xanthium* which commonly occur in the vicinity of Lawrence, Kansas, do not hybridize in the field to any extent because they blossom at different times. "There is a physiological isolation that effectually prevents hybridization in the great majority of cases. The pollen of one variety has been shed long before the stigmas of the other are ready for the pollination processes." Whether such physiological isolation occurs in other localities must be tested in each case.

From the fruits of 42 Montreal specimens, 471 plants were obtained; 250 additional plants developed from the detached burrs which had been collected in the spring. The 107 transplanted seedlings made a total of 828 plants in the garden in the season of 1924. The probability was that these would include all forms of *Xanthium* common in this locality. The first buds appeared early in August on the plants raised from seedlings. In the third week in August, there were blossoms on practically all the plants. There seemed to be no such isolation here as SHULL had found in Kansas.

The recent classification of MILLSPAUGH and SHERFF (11) was used in identifying species. Even with the help of the full discussions and clear plates in this monograph, as well as the original description of each species, some difficulties remained. The form most common on the south shore of the St. Lawrence River was identified as *X. italicum* Moretti. In the key of MILLSPAUGH and SHERFF, *X. echinatum* Murr. and *X. italicum* Mor. are separated by the relation between the length and breadth of the body of the fruit. As the body of the burrs in question was invariably more than twice as long as wide, this point was easily decided. A study of the plates, however, led to the conclusion that the burrs were more like *X. echinatum* in appearance. Moreover, one of the burrs pictured as *X. echinatum* was collected by VICTORIN at Longueuil, where the writer's specimens were gathered. The photograph of this fruit was measured and found to be 17 mm. in length, and 7.5 mm. in breadth, surely more than twice as long as wide. A critical study of the descriptions of *X. echinatum* Murr. and *X. italicum* Mor. as given by MILLSPAUGH

and SHERFF, shows them to be alike in many ways and distinguishable chiefly by measurements of the mature fruit. The leaves and stems of *X. italicum* may attain a considerably greater length than those of *X. echinatum*, but a small plant of the former could not be distinguished in this way. Both have scabrous, purple-spotted

TABLE I

	LENGTH (MM.)			BREADTH (MM.)
	Beaks	Prickles	Body	Body
Average (125)	5.1	4.3	15.9	7.2
Range of variation (125)	4.1–5.9	3.4–4.8	14–18	6.5–8.0
Range X. echinatum	3–5	3–5	16–20	8–10
Range X. italicum	5–7	3–7	13–18	6–8

stems, scabrous leaves which may vary considerably in form, and burrs with body and prickles bearing glands and hairs, and beaks at least partly hispid. Both are reported from Quebec. The beaks of both are described as incurved, although in the case of *X. italicum* the limiting word "plerumque" allows the possibility of their being

TABLE II

	STEM (DM.)	LENGTH OF LEAVES +PETIOLES (DM.)
Range in form studied	6–10	1.3–3.5
Range in X. echinatum	3–6	0.6–2.3*
Range in X. italicum	3–10 (–18)	0.8–3.0*

* Petioles only, about equal to the leaf blades or exceeding these.

straight. In the original description of *X. italicum* (13) the beaks are said to be "linear." The beaks of the writer's Longueuil specimens were sometimes divergent and sometimes incurved.

Measurements were taken of 125 burrs of the species in question. The result is shown in table I, together with the range of measurement given in the descriptions of the two similar species.

Further measurements were taken of stems and leaves (including petioles) and compared as shown in table II. The measurements are seen to conform generally to those expected for *X. italicum* Mor.

Another point omitted from the original descriptions (**13, 14**), as well as from the formal descriptions by MILLSPAUGH and SHERFF, is suggested by the discussion of these authors with regard to *X. maculatum* Rafinesque, which they reduce to synonymy with *X. echinatum* Murr. They quote part of RAFINESQUE'S description, emphasizing in italics the points which lead them to believe that he was describing *X. echinatum*. One of these emphasized phrases is "generally solitary." The fruits of the species occurring commonly at Longueuil are never solitary, so far as the writer has observed, but occur in thick clusters. A similar difficulty was encountered in dis-

TABLE III

	LENGTH (MM.)			BREADTH (MM.)
	Beaks	Prickles	Body	Body
X. pennsylvanicum.....	4–6	3–7	10–20	5–8
X. inflexum.....	5–7	4.5–6.5 (8–10 near beak)	13–17 (–20)	6–7.5

tinguishing *X. inflexum* Mack. and Bush (**7**) from *X. pennsylvanicum* Wallr. Both species apparently occur sparingly here, and may have hybridized to some extent with recombinations of hereditary units. Table III shows the measurement range given for the two species by MILLSPAUGH and SHERFF.

It will be noted that these measurements overlap to a great extent, and unless there are other distinctive characters, there will often be doubtful cases where the two forms occur together. The original description of *X. inflexum* speaks of the beaks as "about 10 mm. long, at maturity abruptly bent at the middle, inflexed and at length overlapping, hooked at the apex." MILLSPAUGH and SHERFF embody all these points in their description, but disagree as to the length of beaks. The beaks of *X. pennsylvanicum* are somewhat variable, according to the descriptions, as to thickness and form. According to MILLSPAUGH and SHERFF, *X. inflexum* is a taller plant than *X. pennsylvanicum*, the former ranging from 10 to 15 dm. in height, the latter from 3 to 9 dm. If the original descriptions are consulted, there is also a difference in the tips of the prickles. WALLROTH (**23**) describes those of *X. pennsylvanicum* as "ending in a short,

lightly bent hook," whereas MACKENZIE and BUSH speak of the prickles of *X. inflexum* as "strongly hooked." These writers do not mention the arcuate character of the spines, which, however, is pointed out by MILLSPAUGH and SHERFF, and which is not a character of *X. pennsylvanicum*. These seem to be the main differences.

The species agree in general stem and leaf characters, and, to a great extent, in the size and shape of the fruit. Both are comparatively smooth as to body and prickles, which bear glands, but few, if any, non-glandular hairs, except at the base of the beaks. Table IV shows the result of measurements of 96 burrs from 8 plants classified as *X. pennsylvanicum*.

TABLE IV

	Length (mm.)			Breadth (mm.)
	Beaks	Prickles	Body	Body
Average (96)	6.1	4.9	18.0	7.0
Range (96)	4.5–9	3.5–7	13–22	5.5–9.5
Range X. pennsylvanicum	4–6	3–7	10–20	5–8

The burr which showed a body thickness of 9.5 mm. was not an exceptional fruit, but grew upon a plant with broadly ovate burrs with an average thickness of 8 mm., few measuring less than 7 mm. thick. This, of course, agrees more closely with *X. pennsylvanicum* than with *X. inflexum*. The prickles, however, were very strongly hooked and arcuate. They ranged in length between 3.5 and 5.5 mm.[1] The beaks in some cases were bent inward from the middle. They measured 5–6 mm. in length. The body length varied between 18 and 20 mm. The body and base of the prickles were thickly dotted with glands. There were few hairs and these almost entirely on the lower part of the beaks. The height of the plants was 7.5–10 dm. These characters were common to the original mother plant and to those grown from its fruits. It will be noted that the arcuate, strongly hooked character of the prickles is typical of *X. inflexum*. The plants also are comparatively tall. This might easily be due to cultivation, but it will be remembered that *X. italicum* did not reach its maxi-

[1] In the discussions, averages concerning progeny of individual plants were usually obtained from measurements of 10 fruits chosen at random.

mum height under the same conditions. The most striking misfit in the table is the beak measurement of 9 mm. This burr was on a plant which had fruits with an average beak length of 7.3 mm., far exceeding normal *X. pennsylvanicum* measurements. The beaks, in some cases, were strongly inflexed. There were instances, also, of the *inflexum* habit of much longer spines near the beaks. These spines, however, did not exceed 7 mm. All the other characters were typical of *X. pennsylvanicum*, and it was therefore classified, for the time, as belonging to the latter species. These two cases, among others noted, seem to support the theory that there has been hybridization, with recombination of characters.

TABLE V

	Length (mm.)			Breadth (mm.)
	Beak	Prickles	Body	Body
Average (40)	6.3	5.6	17.6	6.9
Range (40)	5–9	4.5–7.5	15.5–22	5.5–8.0
Range X. inflexum	5–7 (–10)	4.5–6.5	13–17 (–20)	6–7.5

Table V shows the average and the range found in measuring 40 fruits from 4 plants considered to be *X. inflexum* Mack. & Bush. The beak length of 9 mm. is not too long according to the original description. Evidently the beaks can at least occasionally attain this length.

It has been mentioned in all descriptions of *X. inflexum* that there are occasional longer spines near the beaks. In this species, the average length obtained for prickles depends, therefore, on which ones are measured; 8 mm. is wide for *X. inflexum*, but the original description states only that the body is "2 cm. or less long and less than half that in width." If the proportion is maintained, as it was in all cases observed, the conformity is probably sufficiently close. The other characters of these plants seemed typical of *X. inflexum*. The height of the plants, however, rarely exceeded one meter, and in some cases fell to 6 dm.

The most difficult problem in classification of an apparently pure species was furnished by certain plants collected on the north shore of the St. Lawrence River. The fruits bore a striking resemblance to

those figured by MILLSPAUGH and SHERFF (12) as *X. curvescens*, sp. nov. They had the same stout, divergent, strongly arcuate and hooked beaks, as well as the comparatively few curved and hooked prickles. They were thicker, however, and of a light brown color. While not very hairy, the prickles and beaks were sparsely hispid to about the middle. Among the hairs, as well as distributed over the surface of the body, were numerous glands. The plants, on the other hand, did not at all fit the description in vegetative characters. The stem, far from being "reddish, scabrous, about 3 dm. high," was comparatively smooth and green, usually with green lenticels; the latter, in a few cases, became purple. The plants were tall (9–12 dm.) and grew luxuriantly. Leaves (including petioles) ranged from 1.7 to 3 dm. in length. The blades were larger, thin, and of a light green color, distinct from all the other plants grown. This species was called *X. curvescens*, type *A*. A closely related form was designated type *B*. It had the same general characters as type *A*, but its fruits were less coarse in appearance; the beaks and prickles were shorter, the latter being about twice as numerous. The leaves were firm and of a distinctly darker green. Body and prickles were almost glabrous, although both bore glands. The contrast in foliage between these two types was so striking that fresh leaves were gathered from each, in order to determine, if possible, the reason for the difference in color. An account of this experiment will be given in another section.

Only one plant of type *C* was found. It differed from type *B* chiefly in the hispid character of its body, beaks, and prickles. Its body also had a greater length in proportion to its breadth. Two other plants, of the same general type, had fruits which more nearly fitted the description of *X. curvescens* in regard to their measurements. When these fruits were opened, however, very few seeds were found. This suggested the possibility of type *D*, *X. curvescens*, having arisen apogamously from types *A* or *B*, in which case it would be a haploid form. This point could best be determined by chromosome count, of course, and an attempt was therefore made to obtain material for this. The fact that so many varieties of one species were found supports BITTER'S opinion that the Euxanthia, like the North American species of *Oenothera* of the *biennis* group, are now in a state of intensive separation. Ninety-five fruits of this species, including

types *A*, *B*, and *C*, were carefully measured. Table VI shows the range of variation in size for each type, in comparison with that described for *X. curvescens* MILLSPAUGH and SHERFF. The question arises as to whether this species may be *X. orientale* L. (*X. macrocarpum* DC.).

MILLSPAUGH and SHERFF, in their monograph, show that *X. macrocarpum* DC. was merely a new name created with reason by DE CANDOLLE for *X. orientale* Linn. As a result of their study of the

TABLE VI

MATERIAL	LENGTH (MM.)			BREADTH (MM.)
	Beaks	Prickles	Body	Body
Type A (measurements from 45 fruits from 4 plants)				
Average	5.4	4.6	15.9	6.5
Range	4–7.5	3–7	13–21	5–8.5
Type B (40 fruits from 4 plants)				
Average	4.6	4.0	16.4	6.4
Range	3.5–6.5	3–5	13.5–21.0	5–9
Type C (10 fruits from 1 plant)				
Average	4.9	4.1	17.6	5.9
Range	4–6	3.5–5	16–21	5.5–7
X. curvescens M. & S.	.....	3–6	13–16	3.5–5

species and the literature concerning it, these authorities say, "The true *X. macrocarpum* DC. is a plant with strongly hooked beaks and the prickles somewhat subremote, stoutish, tending to be not only hooked at the apex but also arcuate, often backwardly, then forwardly, from about the middle upward." Discussing specimens of *X. orientale* examined from Europe in comparison with *X. curvescens* from Vermont, they state that in the former they "have found the fruiting involucres to be not only considerably larger but brownish rather than reddish, also much more pubescent and the prickles nearly always more numerous." All this would apply to fruits of the different types of the St. Lawrence River species, and it seems probable that the European species has become established here.

Before any planting had been done, it was thought that *X. Wootoni* Cockerell (ex DE VRIES 5) occurred here. Four plants in the field which bore typical *X. italicum* fruits also bore fruits which would certainly have been classified as *X. Wootoni* if they had been

found as detached burrs (pl. I, *A1*). No plants were found which had only *X. Wootoni* fruits, however, and the *X. Wootoni* burrs planted here produced only the parent type, with occasional fruits like themselves. RYDBERG, of the New York Botanical Garden, kindly allowed the writer to copy a letter written in 1902 from COCKERELL to himself concerning this species. COCKERELL, who discovered it in New Mexico, first found the plants distinct. Later he was somewhat puzzled when he found "on a plant at Las Vegas, a burr of each sort, that is, of *X. Wootoni*, and what we call *X. canadense*, the latter with very crowded prickles." This specimen was regarded as exceptional at the time and placed in the herbarium at Misella Park. Hundreds of both forms, apparently pure, were growing in the same locality. DEVRIES later planted burrs of both kinds sent to him by COCKERELL, and found that they produced plants true to type. In 1913, COCKERELL (3) found in a greenhouse at Boulder a specimen of *X. commune* (now called *X. italicum*) having several *X. Wootoni*-like burrs, although no *X. Wootoni* had ever been seen in Colorado. These duplex specimens reported by COCKERELL seem to have been like the ones found at Montreal. *X. Wootoni* may yet be found here, although the specimens thus far observed seem to be merely varying forms of *X. italicum*. The frequent occurrence of a similar variation under the artificial conditions of the greenhouse, probably comparable with the Boulder specimens, will be discussed later.

Another very striking form, which had fruits varying constantly between two extremes for two generations, has defied the writer's attempts to classify it. The fruits originally collected were fairly uniform in size. Their measurements, as well as body and beak characters, were typical of *X. italicum* Mor. The prickles, however, were generally arcuate and varied greatly in number. The fruits, stripped off and sorted into classes, showed all grades of transition between *X. italicum* and *X. Wootoni*, while many of the prickles were strongly arcuate. The plants grown from these burrs were all alike, and distinctly different in appearance from any other variety in the garden. They were erect and very short, averaging 5–6 dm. The stem had the vertical purple lines characteristic of so many species. The leaves were rather small, thick, and coarsely serrate, a dull, dark green in

color. All plants produced fruits having the same range of variation as the parent, burrs with many and few prickles occurring in the same cluster.

There was a marked difference between the two forms just described, although both were apparently varieties of *X. italicum*, and both produced some burrs of the *X. Wootoni* type. In the one case, there were only the two kinds of burr, as there seem to have been in the specimen found by COCKERELL. In the other, the number of prickles on the fruits varied very greatly, forming a series between the two extremes. The latter differed also in vegetative characters from typical *X. italicum*, and seemed to be an ever-sporting variety.

Another striking case was that of a plant which bore burrs of the two most distinct types of *X. curvescens*, *A* and *B* (pl. IX, *A* 3). They were alike in having only glandular hairs upon body and prickles, and in the arcuate character of their spines and beaks. The coarser type, however, had less than half as many prickles as the other. It was lighter in color and thicker in diameter. Twelve fruits from this plant were sown in the garden and produced fruits of one type, the vegetative characters being those of type *B*. The number of prickles and other fruit characters seemed to be intermediate between the two.

Other types grown by the writer behaved like hybrids, splitting up in the F_2 generation into the parent forms. Mendelian ratios were not obtained exactly, as comparatively few plants were grown from any one parent. For example, 12 fruits from a plant which had all the characteristics of *X. italicum* Mor. produced descendants of two types, about half with burrs of the *X. inflexum* type and the others with fruits having hispid prickles and other characters of *X. italicum*. A second example of what seemed a natural hybrid was furnished by a row of 14 plants, grown from 8 burrs from the same plant of typical *X. pennsylvanicum* character. In the next generation the plants were tall and erect, with smooth green stems and firm scabrous leaves. Some of them, however, bore fruits resembling *X. chinense*, while others had fruits like those of the parent observed. Plants with burrs of the *X. chinense* type were more numerous. Their fruits were small, with few prickles, and characteristic wide open beaks, which were purple-tipped when immature. The latter, usually indicative of the

number of seeds in the burr, varied in number from 2 to 7. The formation of more than the normal number of beaks and seeds was especially characteristic of these *X. chinense* plants, although it occurred to some extent in all plants of this lot. In one case a specimen of each sort came from a single burr.

These cases are suggestive, but it is clear that without controlled pollination Mendelian results can hardly be expected in a locality where several species, between which hybridization is possible, grow and blossom at about the same time. If two forms have crossed in the field, there may be inbreeding in the F_1 generation, but it is possible that pollen from another species or variety will reach at least some of the stigmas. In such a locality as the one here dealt with, where masses of mixed burrs are washed ashore in early spring and germinate close together, many varieties can sometimes be collected within a few yards. It is probable, therefore, that in such spots polyhybrids arise, and that the ovules of a single plant will sometimes be fertilized by pollen from more than one source.

The results of the controlled breeding experiments are summarized as follows. About half of the attempted crosses were successful. In most instances the same cross which failed on one branch succeeded on another. In these cases at least, therefore, the failure was not due to a physiological incompatibility between the species or varieties. The experiments took considerable time. In some cases the pollen had been formed long before it was applied, and in other cases the stigmas may have been too old before pollen was placed upon them. Good seeds were set by all species when the plants were self-pollinated. The following crosses were successful:

1. *X. italicum* crossed with *X. inflexum*
2. *X. italicum* crossed with *X. curvescens A*
3. *X. italicum* crossed with *X. curvescens B*
4. *X. curvescens A* crossed with *X. curvescens B*
5. *X. curvescens B* crossed with *X. inflexum*
6. *X. curvescens B* crossed with *X. pennsylvanicum*

It will be noted that *X. pennsylvanicum* was crossed in only one case. Unfortunately it was found when the fruits matured that this plant of *X. pennsylvanicum* had been heterozygous. The apparent isolation here, however, is not thought to be real. Few crosses were

attempted with this species, the reason being that there was a relatively small number of *X. pennsylvanicum* plants, and few of these were reached while in bud during the time of emasculation and bagging.

Out of more than 50 branches emasculated and left unpollinated, only two were found to have formed burrs to any extent. One of these was of the *X. curvescens A* type. There were 22 apparently normal fruits. These were opened and contained 8 seeds altogether. The other was on one of the heterozygous plants of *X. pennsylvanicum*. Many burrs were opened, but only 9 seeds were obtained. Since there were many branches of each of these types which had been treated similarly and had produced no seeds, it is highly probable that in these two cases a small staminate head had been overlooked in the emasculation, or that a new one had developed later and pollinated these ovules. There were three other instances in which a single seed was found among many fruits opened.

A few of each kind of seed obtained in the breeding experiments were planted in the greenhouse in January, 1925. The main object at that time was to obtain blossoms for cytological study and chromosome count. Owing to lack of space, it was impossible to get any numerical results for genetic conclusions. The burrs were allowed to develop, however, and it was hoped that from them some conclusions might be reached with regard to the heredity of the plants studied. The seeds were planted about 9 in. apart, in boxes 10 in. deep. The house in which they were grown was kept at a temperature of 76° F. during the day. The outdoor temperature was very low at this period, and the temperature inside fell many degrees during the night. The glass had been coated with white lead and no direct sunlight came in. Germination was slow and the seedlings were weak. It was decided, therefore, to try artificial illumination. Thirty-six 75-watt lamps were attached to a frame which was suspended 2.5 feet above the boxes in which the *Xanthium* seedlings were growing. These were kept burning night and day for about two weeks. The plants straightened up and grew more rapidly. A few were injured, however, by an excess of heat. About March 1 the paint was removed from the glass above the plants, and artificial light was no longer used. Buds appeared soon afterwards, and staminate

heads were collected and prepared for cytological study. The fruits are not yet mature (May 8), but the oldest burrs on each plant have reached full size. Some striking abnormalities have appeared. A summary of greenhouse results follows.

X. italicum Mor. (pl. IX, *B1*).—Burrs from seven plants, identified when collected as *X. italicum* Mor., were grown for two generations, the second from seed of plants self-pollinated in the first. In all these cases the mother plants seem to have been homozygous, all the plants obtained showing *X. italicum* characters.

X. pennsylvanicum Wallr. (pl. IX, *B2*).—Two plants of *X. pennsylvanicum* produced burrs of the same type for two generations, although a few fruits with about half the number of prickles were mingled with typical ones on the greenhouse plants.

X. inflexum Mack. & Bush. (pl. IX, *B3*).—Descendants of two plants of *X. inflexum* were observed for two generations. By the first, *X. inflexum* characters were produced for the most part in both, although the beaks were not bent at the middle in the characteristic way on the greenhouse plants. The other produced *X. italicum* in the second generation, which seemed to indicate that the original plant had been heterozygous.

X. curvescens, type *A* (pl. IX, *B4a*).—Burrs from four plants, identified when collected as *X. curvescens*, type *A*, were grown for two generations in the same way. The first summer all the plants produced fruits of the same kind as those on the parent plant. In the generation grown in the greenhouse, the habit of the plants was of the same nature, and the general shape and size of fruits were the same, but the very striking arcuate character of both prickles and beaks seemed to have been lost.

X. curvescens, type *B* (pl. IX, *B4b*).—Burrs from three plants of *X. curvescens*, type *B*, reproduced the original characters when grown in the garden. Only one of these, selfed and grown in the greenhouse, produced characteristic fruits with incurved beaks and many arcuate prickles. On the same plant were a few abnormal burrs of the *X. Wootoni* type. A second, selfed, produced burrs which fitted the description of *X. pennsylvanicum*. Fruits of the third resembled the original type except in their beaks, which were short, stout, and not incurved.

X. curvescens, type *C* (pl. IX, *B4c*).—Type *C*, selfed, produced only fruits of the abnormal type, with very few prickles and these not curved. The shape and pubescent character of the fruit, however, and the character of the beaks were true to type.

X. curvescens, type *D* (pl. IX, *B4d, e*).—There were very few seeds from the two plants of type *D*. Six were planted and two germinated. Pollen was transferred from one to the other in both directions, and a few burrs were formed on each. In one case they were true to type, in the other a new type appeared with exceedingly long spines and no good seeds. One seed from the former plant was sown in the greenhouse but did not germinate.

HYBRIDS.—Two seeds of each hybrid artificially produced were sown in the greenhouse. Pl. X shows typical specimens. From the genetic viewpoint only very general conclusions can be drawn from the greenhouse results. So far as normal fruits were obtained, it seemed that *X. italicum* qualities were dominant to those of *X. inflexum*, *X. pennsylvanicum*, or any of the types called *X. curvescens*. *A* and *B curvescens* crossed gave fruits of the *A* type. Unfortunately, the plants obtained by crossing *X. curvescens* with *X. pennsylvanicum* died in the seedling stage.

On nearly all the greenhouse plants there were some abnormal burrs, of which the plates show good examples. In every species grown there was occasional reduction in number of prickles, giving rise to fruits of the *X. Wootoni* type. It seemed possible that bud-sporting was common in the genus, and that the tendency was emphasized under the artificial conditions in the greenhouse. A case of fasciation which occurred on a plant of *X. italicum* in the garden, affecting one of the two lowest branches, suggested this explanation. It was found, however, that the abnormal burrs were often scattered here and there in clusters of normal fruits. The fact that the staminate heads had been removed from the tips of the flowering branches suggested that the wounding of the tissue had induced the formation of abnormal burrs. There were clusters on some plants, however, which had not been disturbed and which still bore *X. Wootoni* burrs. Further investigation is necessary before definite conclusions are reached with regard to the cause of this phenomenon. A number of possible experiments under natural and controlled conditions suggest

themselves in this connection. While the greenhouse environment was thought to be unsuitable for obtaining reliable genetical data, possibilities which might otherwise have been overlooked have been revealed as a result of it.

2. Cytology

It was thought that specific differences in *Xanthium* might be correlated with differences in chromosome number, as has been found to be the case in *Datura*, *Oenothera*, *Rosa*, and many other genera. Slides showing pollen mother cells in different stages of division have been studied in *X. italicum*, *X. pennsylvanicum*, and *X. inflexum* (the last probably heterozygous), as well as in two hybrids which were obtained by controlled pollination. As has already been explained, it has not been possible in the time thus far devoted to the investigation to prove, without doubt, the purity of the species under consideration. A preliminary survey of the chromosomes in the forms studied, however, would seem to indicate that the difference between species of *Xanthium* is probably a difference in factors, rather than a difference in chromosome number. The reduced number, in all the cases studied, was 18. Pl. XI *X* will suffice to show the size and general appearance of the chromosomes in the heterotypic metaphase. The five upper cells were from hybrid *X. inflexum* × *italicum;* the four below from *X. pennsylvanicum.* The drawings were made with the camera lucida and are shown in the photograph magnified 1220 diameters.

3. Physiology

As is well known, the two seeds in the normal *Xanthium* fruit differ in size. The smaller one is more protected by the inclosing burr. It was long ago observed that the larger, lower seed tended to germinate first. Rowlee (16), in 1893, mentioned the difference in the time of germination of the two seeds, and much has since been written upon the subject. As a result of the researches of Crocker, Shull, and Davis, reported at intervals since 1906, the factors responsible for delayed germination in *Xanthium* have largely been determined. An experiment in the McGill greenhouse during the winter of 1921 suggested that the determining differences between upper and lower seeds might not be equally great in all species of

Xanthium. This experiment involved about 150 burrs, which were sorted and planted in flats. Within about 10 days, one plant had been produced by most of the burrs. One type, however, consistently showed two seedlings from each fruit. It was therefore decided to make further observations with regard to this point, and general field notes upon delayed germination.

CROCKER (4) had pointed out that the burr was indirectly responsible for much of the delay in germination of the upper seed. Having shown that the coats of the upper seeds contribute to the delay by excluding more oxygen than those of the lower seeds, he says that the growth of the former in nature "comes about by a partial disintegration of the seed-coats. . . . . The length of the delay depends upon the ability of the seed-coat, protected by the surrounding burr, to resist the factors of disintegration in the soil. The portion of the burr covering the lower seed decays within a few months after burial, while the portion covering the upper seed is always far more persistent." More recently, MCHARGUE (9) has laid even greater stress upon "the mechanical conditions within the burr," arguing that these are sufficient to cause all the delay observed in his experiments.

In the summer of 1923 half the burrs were planted whole and half were opened. This gave equal numbers of whole burrs, lower and upper seeds, except where one seed was found to be lacking or was injured in the process of removal. The net result was as follows: Of 367 whole burrs planted 136 produced no plants that year (about 37 per cent), 220 produced one plant each (about 60 per cent), 11 produced two plants each (about 3 per cent). Of 278 lower seeds planted 221 produced plants (about 79.5 per cent), 57 produced no plants (about 20.5 per cent). Of 279 upper seeds planted 93 produced plants (about 33.3 per cent), 186 produced no plants (about 66.7 per cent).

SHULL (21) and others have pointed out that weeds generally have a very small percentage of non-viable seeds. The fact that 37 per cent of the whole burrs planted produced no plants in that season is partly to be accounted for by the prevalence of the *Xanthium* trypetid fly, especially in burrs from the south. When the burrs were opened for the removal of seeds, a very large proportion of them were

found to contain pupae of various ages. A number of these pupae were removed without injury and placed upon damp filter paper in a covered preparation dish. Two males and a female emerged and were identified by WILLEY as *Trypeta aequalis* Loew. This insect was discussed by MARLATT (8) in 1891 as a natural enemy of *Xanthium*, of wide distribution and of possible economic importance. He described the ecological relation between insect and plant, and observed: "The larva has never been known to occur in more than one

TABLE VII

No.	Source	Both good	Both spoiled	Lower good; upper spoiled	Upper good; lower spoiled	Upper			Lower		
						Good	With pupae	Shriveled	Good	With pupae	Shriveled
100..	Georgia	42	17	11	30	72	5	23	53	12	35
100..	Alabama	37	13	10	40	77	6	17	47	30	23
100..	Florida	28	12	6	54	82	10	8	34	39	27
50..	South Carolina	29	2	5	14	43	1	6	34	8	8
50..	North Carolina	37	1	6	6	43	1	6	43	5	2
325..	Ohio	256	6	27	36	292	4	29	283	11	31
50..	Montana	25	7	8	10	35	7	8	33	11	6
100..	Kansas	44	7	20	29	73	6	21	64	13	23
50..	Illinois	40	3	2	5	45	0	5	42	1	7
75..	Wyoming	33	16	12	14	47	12	16	45	11	19
1000		571	84	107	238	809	52	139	678	141	181
Percentage		57.1	8.4	10.7	23.8	80.9	5.2	13.9	67.8	14.1	18.1
Probably		57.1	8.4	10.7	23.8	80.9	19.1		67.8	32.2	

of the two seeds normally contained in the *Xanthium* burr." Abundant material being on hand, it was thought that it would be of interest to determine (1) to what extent the burrs had been attacked by this insect, (2) whether the insect ever destroyed both seeds, (3) whether the insect showed any preference for the larger lower seed. A thousand burrs from the same stock used for planting, which included six different types from ten different states, were opened and examined with a view to deciding these points. The results are summarized in table VII.

When both seeds were spoiled, it was probably in most cases due to two insect punctures, one penetrating each ovule. Several times two well developed pupae were found in one burr, each entirely fill-

ing one seed cavity. Usually only one puncture was made in a burr. The lower seed was more often destroyed in this way than the upper, but there was no evidence that the insect follows a regular course of action in this respect. The fact that more than 32 per cent of the lower seeds were here found to be spoiled, helps to account for the low percentage of germination from whole burrs, already noted. The fact that one-fifth of the lower and two-thirds of the upper seeds planted failed to produce plants is still to be explained. Many of these failures were in the southern forms (*X. chinense* Mill. 10). These have small seeds, and it is probable that they were planted too deeply. Later, when this was suspected, part of the earth was removed in some of the rows and more seeds then germinated. The fact that under the same conditions a much larger percentage of lower seeds germinated supports the findings of CROCKER and SHULL (18, 19, 20). One-third, however, of the 279 upper seeds planted did germinate without delay.

SHULL and DAVIS (21) discuss possible reasons for the fact that MCHARGUE did not find the normal percentage of delay in the first year. Of the four suggested possibilities, the only one which might apply to this case is that the coats may have been injured by handling. Care was taken not to break the seed or cut the seed coat. Many seeds were prepared for planting at one time and wrapped in soft paper, however, the ends of each small package being labeled. When opened and planted, the coats were apparently intact; 33 per cent of the upper seeds thus planted germinated within about 10 days, while in the same period only 3 per cent of the whole burrs planted produced two seedlings each. This difference could hardly all be accounted for by the factors noted. It seems clear from this, and from the next year's results, that the burr factor is an important one in causing delay in nature.

In 1924 most of the burrs were planted whole; in one part of the garden seeds from opened burrs were sown. The records of planting were not kept in the same way as in 1923, but the general result was noted. There was a marked difference in the germination percentage of burrs and seeds gathered in the fall and stored in the laboratory until planting time, and that of those gathered on the shore in the spring. Of the latter, 213 burrs were planted, 67 of them producing

two plants each. Of the former, 442 burrs and seeds were planted; 45 burrs produced two plants each, while 26 upper seeds from opened fruits also germinated. More than 30 per cent of the burrs which had been exposed to outside conditions during the winter produced two plants each. Of the ones which had been stored dry, most of those which produced two seedlings were *X. pennsylvanicum*. In one case, from a single plant of this species, 11 burrs were sown and 8 of them produced two equally strong plants.

McHARGUE says, "Plants from large seeds are in every respect larger and more vigorous; this difference maintains after 60 days."

TABLE VIII

	PLANTS EQUAL	ONE PLANT SLIGHTLY LARGER; BOTH STRONG	ONE PLANT DISTINCTLY STRONGER
Separated.....	69	34	11
Unseparated.....	13	7	1
Total.....	82	41	12

Under the conditions in the garden this was not generally the case. Two hundred and seventy paired plants, from 135 fruits, some of them from the miscellaneous burrs washed ashore and gathered in the spring, and others from burrs from known mother plants, were kept under observation during the summer. In the fall they were compared as to size, productiveness, and other characters. Twenty-one of these had been left unseparated throughout the season. All 270 plants were strong, with branches in every leaf axil and abundance of fruit. Numerical results are given in table VIII.

While the duplex seedlings occurred most frequently in *X. pennsylvanicum*, they were produced occasionally by other species. They were seldom observed in *X. italicum*. Generally, however, the tendency was seen in a number of burrs from one parent, rather than in isolated cases. It seemed to be a varietal difference.

BITTER grew *X. italicum* under observation and separated three races by their stem color, with which he found correlated other physiological differences; notably the time of flowering varied directly with the amount of color in the stem. Differences in stem color were

also noted by the writer among plants of *X. italicum* from different parents. The general habit, especially the angle of branching, also varied.

Another varietal difference which has already been mentioned, was that of the leaf color in types *A* and *B* of *X. curvescens*. Leaves of each kind were gathered and examined in section under the microscope. No very great difference in structure being noted, it was decided to extract and compare the pigments. Leaves were dried and powdered, and equal weights of the two kinds were taken. The directions of ONSLOW (15) were followed in the extraction and separation of the pigments. The latter were then compared with a colorimeter, at least 20 readings being taken and averaged in each case. It was found that type *B* leaves contained more of chlorophyll *a*, carotin, and xanthophyll, while there was slightly more of chlorophyll *β* in the lighter green leaves of type *A*. The ratios were as follows:

	A	B
Chlorophyll *a*	75	100
Chlorophyll *β*	100	96
Carotin	65	100
Xanthophyll	85	100

As the growing plants have been observed during the past two years, many new problems have presented themselves. Some of them have been indicated in this paper. The writer hopes to continue the investigation and to obtain further results along these lines.

Summary

1. Species of *Xanthium* found commonly on the shores of the St. Lawrence River at Montreal are *X. italicum* Mor., *X. pennsylvanicum* Wallr., *X. inflexum* Mack. & Bush, and a form thought to be *X. orientale* Linn., but of the described American species most like *X. curvescens* Millsp. & Sherff.

2. These species all blossom at about the same time, and experiment has shown that hybridization is possible between them.

3. Plants of *X. italicum* with some burrs of the *X. Wootoni* type have been found wild here. They fit the description of the "duplex specimen" found by COCKERELL in New Mexico.

4. Plants of all the species studied and some hybrid forms, when forced in the greenhouse, produced some *X. Wootoni* burrs.

5. In hybrids, the characters of *X. italicum* apparently are dominant to those of any of the other three species grown here.

6. In the species and hybrids thus far investigated, the reduced chromosome number was found to be 18.

7. In garden observations upon delayed germination during two summers, it has been found that the burr is an important factor in causing delay in nature.

8. MARLATT's suggestion that *Trypeta aequalis* Loew is an important natural enemy of *Xanthium* has been confirmed by observations upon 1000 burrs from the southern United States. It was found that sometimes both seeds in a burr were attacked by the insect, although it was usually only one, and more often the lower.

9. In the garden, when both seeds in a single burr germinated, the two seedlings were often equal in size and strength, while only rarely was one much more vigorous than the other.

10. The tendency to produce two plants the first season seemed to be characteristic of some varieties. Other varietal differences were noted among progeny of different plants of the same species.

The writer wishes to express thanks to Professor F. E. LLOYD for his constant interest, and for his aid in photographing the specimens; to thank Professor C. M. DERICK for valued suggestions and encouragement, Dr. J. N. ROSE under whose personal direction the facilities of the National Herbarium at Washington were enjoyed, the Directors of the Agricultural Experiment Stations in the United States and Canada, and all who cooperated in the collection of material.

McGILL UNIVERSITY
MONTREAL, CANADA

LITERATURE CITED

1. ALLEN, EZRA, Cell division in the albino rat. Anat. Rec. Phila. **10**:576–577. 1916.
2. BITTER G., Über Verschiedenheiten in der Entwicklungsdauer bei *Xanthium* Rassen. Abhandl. Nat. Ver. Bremen **19**^{II}:291–297. 1908.
3. COCKERELL, T. D. A., Science N.S. **42**:871. 1915.

4. CROCKER, W., Rôle of seed coats in delayed germination. BOT. GAZ. **42**: 265–291. 1906.
5. DE VRIES, H., Species and varieties. p. 140. 1905.
6. LASCH, W., Drei *Xanthium* Arten mit ihren Bastarden. Bot. Zeit. **14**:409–415. 1856.
7. MACKENZIE, and BUSH, Rept. Mo. Bot. Gard. **16**:106. 1905.
8. MARLATT, C. L., The *Xanthium* Trypeta. Insect Life **3**:312–313. 1891.
9. MCHARGUE, J. S., Some points of interest concerning the cocklebur and its seeds. Ecology. **2**:110–119. 1921.
10. MILLER, PHILLIP, Gard. Dict. ed. 8. X. no. 4. 1768.
11. MILLSPAUGH, C. F., and SHERFF, E. E., Revision of the North American species of *Xanthium*. Field Mus. Publ. Bot. 4. 1919.
12. MILLSPAUGH, C. F., and SHERFF, E. E., *loc. cit.* 25. 1919.
13. MORETTI, Brugnat. Giorn. Fis. Chim. Dec. II. **5**:326. 1822.
14. MURRAY, Com. Gött. **6**:32. 1784.
15. ONSLOW, M. W., Practical plant biochemistry. 1920.
16. ROWLEE, W. W., Studies upon akenes and seedlings of plants of the order Compositae. Bull. Torr. Bot. Club **20**:10. 1893.
17. SHULL, C. A., Physiological isolation of types in the genus *Xanthium*. BOT. GAZ. **59**:474–483. 1915.
18. ———, Oxygen pressure and the germination of *Xanthium* seeds. BOT. GAZ. **48**:387–390. 1909.
19. ———, The oxygen minimum and the germination of *Xanthium* seeds. BOT. GAZ. **52**:453–476. 1911.
20. ———, The rôle of oxygen in germination. BOT. GAZ. **57**:64–69. 1914.
21. SHULL, C. A., and DAVIS, W. B., Delayed germination and catalase activity in *Xanthium*. BOT. GAZ. **75**:268–281. 1923.
22. TAYLOR, W. R., The smear method for plant cytology. BOT. GAZ. **78**:236–238. 1924.
23. WALLROTH, Beit. Bot. 1^{II}:236. 1844.

DESCRIPTION OF PLATES IX–XI

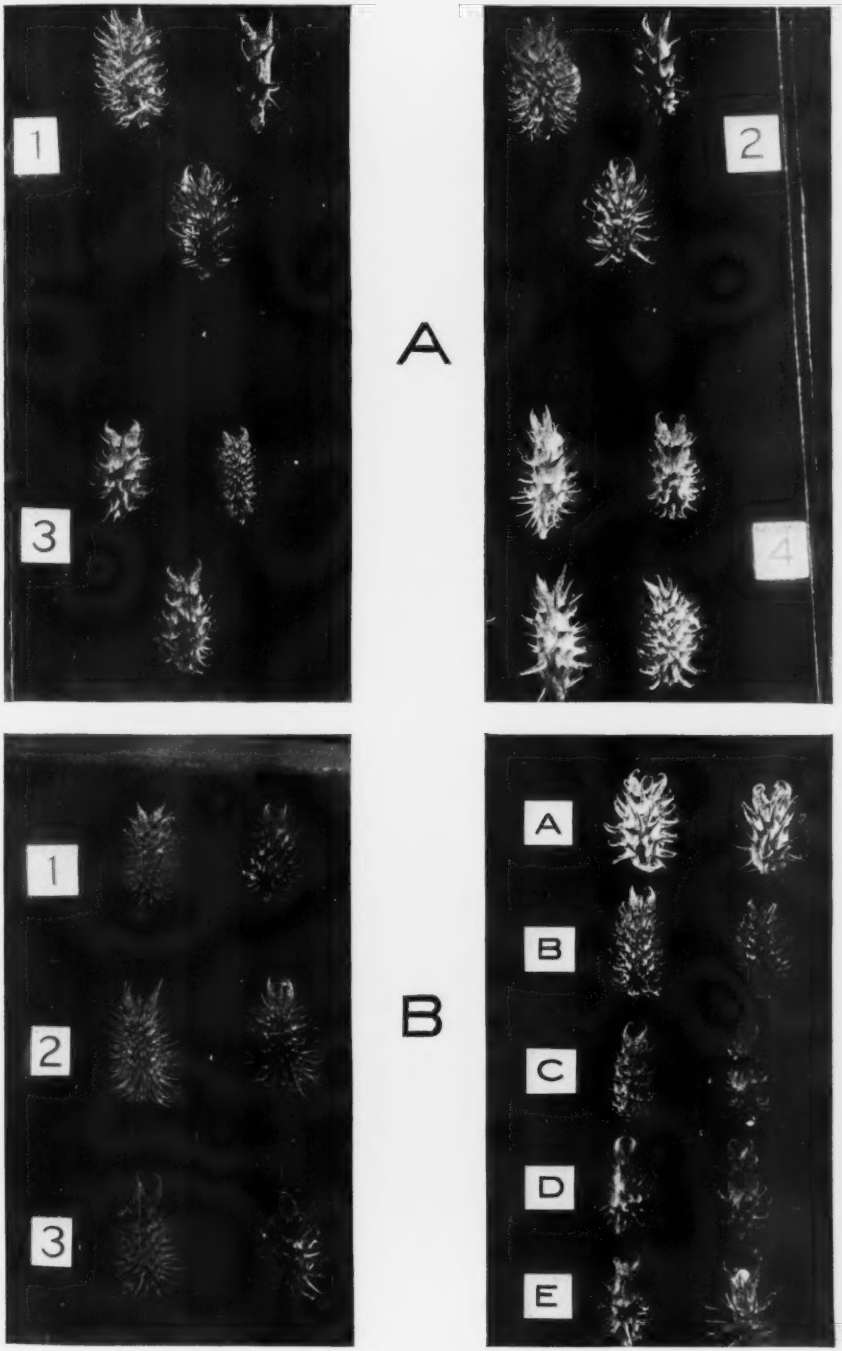
PLATE IX

A: 1, 2, 3, 4.—Above, fruits of different character found on same plant; below, typical fruit or fruits produced in next generation by seeds from burrs of both kinds.

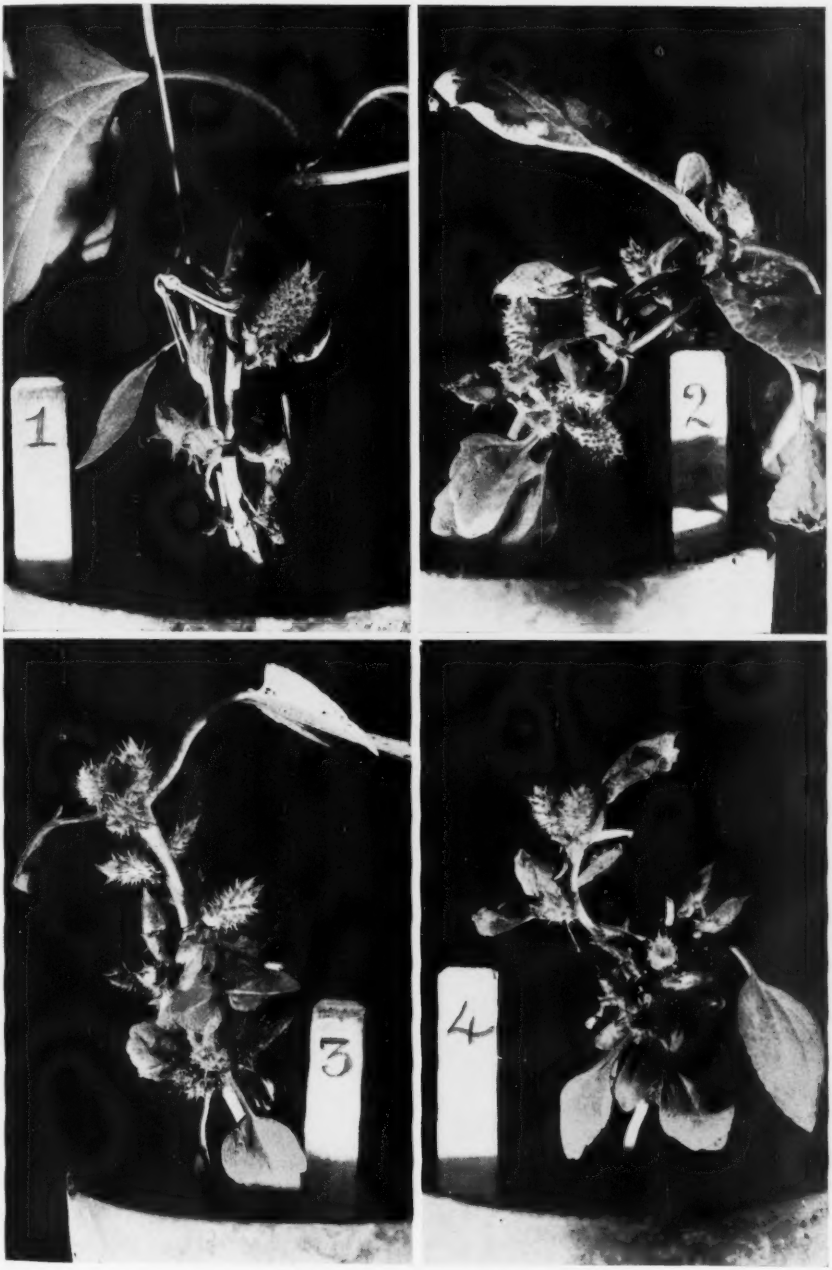
B.—1, *X. italicum* Mor.; 2, *X. pennsylvanicum* Wallr.; 3, *X. inflexum* Mack. & Bush; types a, b, c, d, e, *X. curvescens* Millsp. & Sherff.

PLATE X

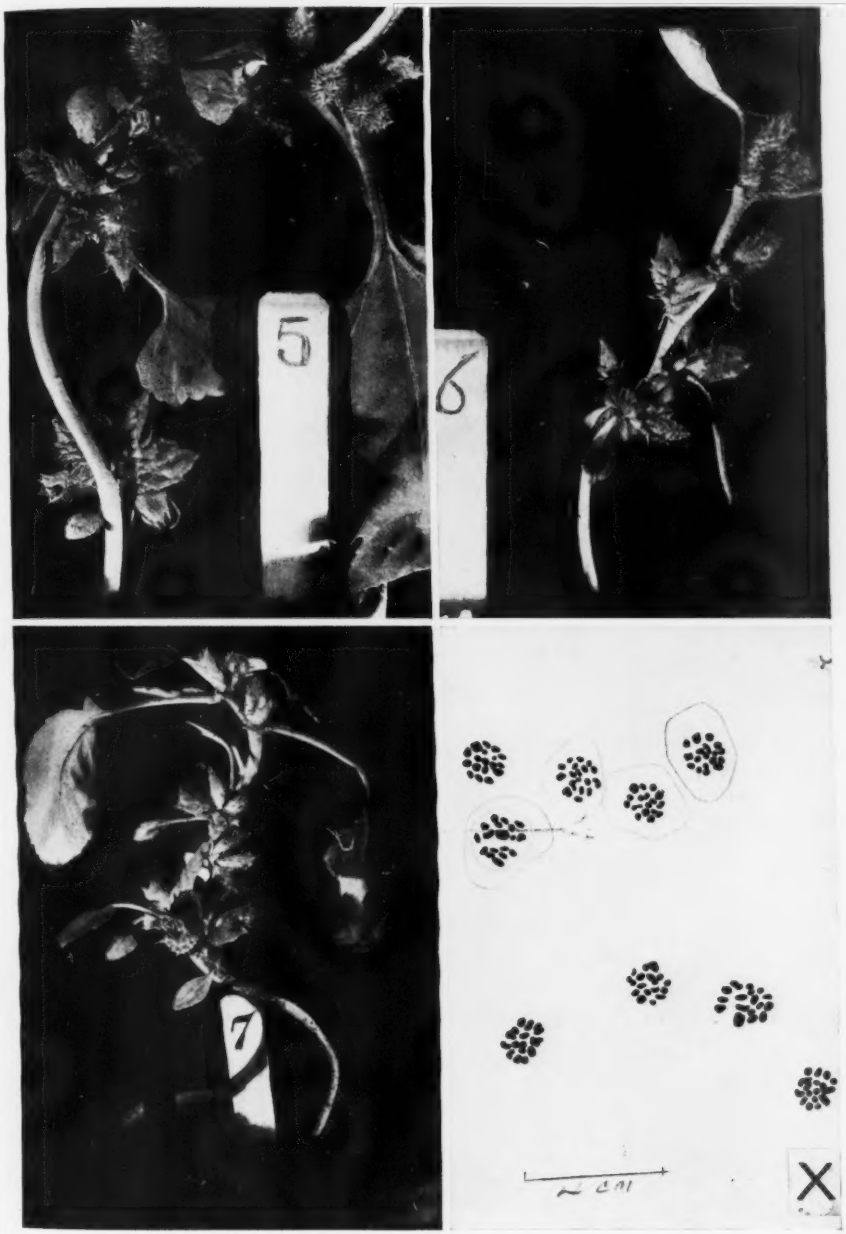
FIG. 1.—Hybrid obtained by crossing *X. italicum* and *X. inflexum:* only two fruits were left to develop; upper one a typical *X. italicum* burr, lower one quite abnormal.



SYMONS on XANTHIUM



SYMONS on XANTHIUM



SYMONS on XANTHIUM

FIG. 2.—Hybrid obtained by crossing a variable form of *X. italicum* and *X. pennsylvanicum:* normal fruits of *X. italicum* type.

FIG. 3.—Hybrid obtained by crossing *X. italicum* and *X. inflexum:* normal burrs have *X. italicum* characters (parent plants different from those used in fig. 1).

FIG. 4.—Hybrid obtained by crossing *X. italicum* and *X. curvescens B*: lower clusters show burrs resembling *X. Wootoni*, while rather small *X. italicum* fruits are seen at top of plant.

PLATE XI

FIGS. 5, 6, 7.—Plants of pure species, selfed, which produced burrs of *X. Wootoni* type when grown in greenhouse; fig. X, chromosomes in heterotypic metaphase; magnification 1220 diameters; above, five pollen mother cells from hybrid *X. inflexum* × *italicum;* below, four pollen mother cells from *X. pennsylvanicum.*

POLLEN DEVELOPMENT IN THE APPLE, WITH SPECIAL REFERENCE TO CHROMOSOME BEHAVIOR[1]

J. S. SHOEMAKER

(WITH PLATES XII–XIV)

Introduction

Fruit development in the apple is dependent in large degree upon successful pollen development and subsequent fertilization. The quality of pollen available for fertilization may affect not only the fruitfulness of existing varieties, but also may influence the germinal constitution of the progeny. Investigators, however, have been concerned more with pointing out abnormalities affecting fruitfulness in the apple than with attempting to determine underlying causes.

A study of male gametogenesis of certain varieties in which a high percentage of normal pollen is found, and of others where indications of abnormal pollen development are outstanding, should show the time and sequence of manifested irregularities, and furnish a basis of interpretation of the possible causes. The varieties of the Winesap group (Winesap and its seedlings) are noted for self, inter, and cross sterility, and are characterized further by a low percentage of pollen germination. On the other hand, Delicious pollen is of high germination capacity and is very successful in cross fertilization. The purpose of this study is to investigate chromosome behavior with reference to pollen development in Delicious and in certain members of the Winesap group.

The status of the problem is indicated by a review of previous investigations on the relation of size of pollen grain to viability, the comparative value of Delicious and Stayman Winesap pollen in controlled pollination studies, and chromosome number and behavior in Rosaceae.

[1] Published with the approval of the Director, as Paper no. 546 of the Journal Series of the Minnesota Agricultural Experiment Station. Also submitted to the Faculty of the Graduate School of the University of Minnesota, June 1925, as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

Relation of size of pollen grain to viability

Germination studies of apple pollen were conducted by BEAUMONT and KNIGHT (5), who found that pollen grains which did not germinate in artificial media could be divided into the following four classes:

1. Shrunken and shriveled grains without apparent cytoplasm.

2. Small round grains with cytoplasm, but having a diameter of only one-half to three-fourths the diameter of normal pollen grains. These swell slightly and occasionally burst.

3. A small class of grains which average much larger than the normal grains and are filled with cytoplasm. These grains do not ordinarily produce tubes, or if they do so the tubes are abortive.

4. Grains normal in size and apparently in contents, which produce tubes that burst after attaining a length of approximately two pollen grain diameters.

TABLE I

AVERAGE PERCENTAGES OF POLLEN GERMINATION OF THREE MEMBERS OF WINESAP GROUP AND DELICIOUS

VARIETY	TOTAL NUMBER OBSERVATIONS	AVERAGE PERCENTAGES POLLEN GERMINATION
Paragon*	29	28.3
Stayman Winesap	37	36.9
Winesap	17	65.5
Delicious	38	88.5

*This variety was purchased from a nurseryman as Gilbert Winesap, and was mentioned by BEAUMONT and KNIGHT as such, but has later been identified as Paragon.

The average percentages of pollen germination of various apple varieties also were studied by BEAUMONT and KNIGHT, and considerable variation was found. The average percentages of germination denoted differences not only between individual varieties, but also between the members of the Winesap group and Delicious. Table I, adapted from BEAUMONT and KNIGHT, indicates the average percentages of pollen germination of Paragon, Stayman Winesap, Winesap, and Delicious.

The studies and data of BEAUMONT and KNIGHT indicate three salient features pertinent to the present investigation: (1) that apple pollen grains can be divided into distinct classes according to size; (2) that there is some correlation between size and viability of pollen

grains; and (3) that the percentage of pollen germination of the members of the Winesap group is considerably lower than that of Delicious.

Comparative value of Delicious and Stayman Winesap pollen in controlled pollination studies

The low and high average germination percentages previously mentioned for Stayman Winesap and Delicious suggest that certain internal factors may have influenced the results. This point is

TABLE II

PERCENTAGE SET OF FRUIT WITH BOTH DELICIOUS AND STAYMAN WINESAP USED AS POLLEN PARENTS IN CONTROLLED CROSSES

POLLEN VARIETY	STATION	PERCENTAGE SET OF FRUIT
Delicious	Minnesota	9.28
	Washington	7.09
	Ohio	20.01
		Average 12.13
Stayman Winesap	Minnesota	3.34
	Washington	0.00
	Ohio	6.60
		Average 6.31

emphasized by a consideration of the comparative value of the two varieties as pollen parents in pollination work. Investigations conducted by POWELL (31) indicated that Stayman Winesap and Paragon were both self and inter sterile. Further tests by CLOSE (9), BALLARD (4), GOWEN (19), DORSEY (14), MORRIS (29), AUCHTER (2, 3), KEIL (21), and WILLIAMS[2] (43) showed that the members of the Winesap group were self and inter sterile. Pollination data from various stations, where both Delicious and Stayman Winesap were used as male parents in controlled crosses, have been summarized from reports of DORSEY, MORRIS, KIEL, and WILLIAMS, and are given in table II.

At these stations the pollen of Delicious was used on 19 varieties and that of Stayman Winesap on 17. It is evident from the data

[2] Director's Annual Report.

presented in table II that in a relatively great number of pollinations on many varieties, and under approximately similar conditions, Delicious as a pollen parent was considerably more successful than Stayman Winesap.

Chromosome number and behavior in Rosaceae

Workers have investigated chromosome number and behavior in Rosaceae rather extensively, and have reported numbers for a few economic fruits of the family. In the strawberry, VALLEAU (40) found the haploid number to be 26. In the plum, DORSEY (12) reported 10 haploid chromosomes for a number of species and species hybrids. KNOWLTON (24) stated that in the J. H. Hale peach, a self sterile variety in which a high percentage of aborted pollen occurred, the haploid chromosome number was 8. Investigations in *Rubus* by LONGLEY (25) and LONGLEY and DARROW (27) showed that the basic number was 7 for this genus. In *Potentilla rupestris* and *P. sylvestris* haploid numbers of 8 and 16, respectively, were found by FORENBACHER (in WILSON 44). MURBECK (in WILSON 44) believed that the haploid number in *Alchemilla arvensis* and *A. grossidens* was 16. In *Crataegus monogyna*, MEYER (28) found the haploid chromosome number in the pollen mother cells to be 16. In a study of many species of *Crataegus*, LONGLEY (26) observed gametophytic chromosome numbers of 16, 24, and 32. In *Rosa*, TÄCKHOLM (38, 39), BLACKBURN and HARRISON (7), and PENLAND (30) found 7 as the fundamental haploid chromosome number for the genus.

From a survey of the preceding genera of Rosaceae, it is apparent that they may be grouped into three classes with respect to chromosome numbers: (*a*) with 8, or multiples of it, as the haploid number (peach, *Potentilla* spp., *Alchemilla* spp., and *Crataegus* spp.); (*b*) with 7 as the basic number (*Rosa* and *Rubus*); and (*c*) with neither 7 nor 8 as the basic number (plum and strawberry).

In triploid, pentaploid, and hexaploid forms of *Rosa*, BLACKBURN and HARRISON observed that in many cases the split univalent chromosomes failed to reach the poles, and formed micronuclei. They believed that hybridity in *Rosa* was evident from the irregular chromosome distribution and resultant abnormal pollen

grains. PENLAND found in the diakinesis stage of *Rosa*, that besides a certain number of bivalent chromosomes, many univalents were present also. He thought that this might be due to the addition of parental chromosomes, or to the partial conjugation of unequal sets of chromosomes. According to him the probable explanation of these conditions was incompatibility, either quantitative or qualitative. PENLAND furthermore showed that as early as the anaphase it was apparent that the unpaired chromosomes had either lost their motility, or else were not subject to the same forces as the bivalents, for they were found free from it and out in the cytoplasm. Sooner or later, however, the majority of them became massed about the equatorial plate, following the bivalents (somewhat later) into the telophase, thus giving an anaphase two distinct steps. As a result of this lagging, many of the univalent chromosomes were left outside the membrane of the daughter nuclei. In subsequent divisions of the pollen mother cell they were found to produce separate spindles of their own. TÄCKHOLM (**39**) concluded from studies of *Rosa* that the chromosome conditions found in the polyploid forms indicated hybrid origin. He believed that pollen sterility in *Rosa* was the outcome of latent hybridity, and that pollen grains which received bivalent and a few univalent chromosomes were viable.

LONGLEY (**26**) found that the species of *Crataegus* could be divided into three major classes : (1) diploid species characterized by normal pollen formation; (2) triploid and tetraploid species that were able to develop their pollen mother cells to the tetrad stage (these showed irregularities in their chromosome distribution that frequently led to polyspory and polycary); and (3) triploid and tetraploid species in which the pollen mother cells had thin and vacuolated cytoplasm and seldom developed to the tetrad stage (these represented hybrids that had resulted from crosses of distantly related species). The characteristics shown by these polyploid forms, namely, pollen sterility, irregular chromosome distribution, polyspory, and polycary, were considered by LONGLEY to be features associated with hybrid forms.

A study by LONGLEY (**25**) of pollen mother cells of various species and forms of *Rubus* indicated that they could be divided into

two major classes, according to chromosome number and behavior: (1) diploid, in which the gametophytic number was 7; and (2) polyploid, including triploid, tetraploid, pentaploid, hexaploid, and octaploid, in which haploid chromosome numbers of 14 and 21/2 occurred. Many of the New England species of *Rubus* were found by him to belong to the triploid group. The diploid class was characterized by normal distribution of chromosomes and pollen formation, and the polyploid class by striking irregularities in chromosome behavior and pollen development. In a later paper, LONGLEY and DARROW (27) discussed the irregular distribution of chromosomes in the polyploid raspberry variety La France. During the reduction division some of the chromosomes lagged on the spindle and were extruded into the cytoplasm, and remained outside the daughter nuclei. The resultant pollen grains varied in chromosome numbers and in viability, which they believed to be indicative of hybrid origin. The same investigators reported a second group of sterile raspberries including all but two of the polyploidous forms. At least six forms were sterile and were characterized by an unequal distribution of the chromosomes in tetrad formation. They believed that sterility in these forms might be attributed to an unbalanced chromosome condition in the nuclei of the pollen grain.

The study herein reported was made to determine the chromosome number of certain apple varieties, to trace the chromosomes through the various stages of normal gametogenesis, and to investigate abnormalities which occur in pollen development.

Methods

Three varieties of apples were selected for cytological study of pollen development, Stayman Winesap, Paragon, and Delicious. Twigs of these were taken from the orchard of the Minnesota State Fruit Breeding Farm, Zumbra Heights, and forced in a greenhouse at University Farm, St. Paul. Additional material was collected directly in the orchard. Buds of each were killed at intervals from the time of the first swelling until the individual flower buds of the cluster were fully formed. Each bud was separated from the cluster and the epidermal hairs removed in order to facilitate fixation and subsequent cutting. ALLEN'S (1) modification of Bouin's solution

was used as the killing and fixing fluid. This gave good results in nearly all stages. In the early spireme and synapsis stages, however, there seemed to be a tendency for the chromatin to become disorganized with this fixative. The material was prepared for study by the paraffin method, stained with Haidenhain's iron-haematoxylin, and cut with the microtome at thicknesses within the range of 5–12 μ.

Pollen grain measurements of the three varieties were made at the Fruit Breeding Farm. Anthers with long filaments were snipped off and the pollen teased out in drops of lactic acid on 25×75 mm. glass slides. The lactic acid was employed to afford a liquid medium which would prevent bursting and germination of the pollen grains. The pollen grains of each variety were outlined with the aid of the camera lucida, measured in millimeters, and later calculated to their proper size in microns. The greatest diameter, usually from a germ pore to the opposite side of the triangular pollen grain, was considered as the basis of measurement. In grains which were more round than triangular this method could generally be followed. One thousand pollen grains of each variety were measured and grouped into classes according to diameter.

Normal pollen development

Pollen mother cells

In a given loculus of an anther the pollen mother cells of the apple show little variation in stage of development, although some are slightly more advanced than others. Different loculi of the same anther, however, frequently attain different stages of development, and the variation between loculi of other anthers of the same flower is still greater. Thus within a section of a flower stages ranging from resting pollen mother cells to tetrads may be observed. Within the cluster of buds a greater range is found, the central leader being considerably more advanced than the laterals. Gates (16) believed that irregularity in stages of development of cells in the loculi probably was connected with the failure in pollen development in forms of *Oenothera*.

In material killed April 24, 1924, the sporogenous cells of the anthers were in the rounded resting pollen mother cell stage (fig. 1), and had evidently passed the winter in this stage or just previous

to it. The pollen mother cells are easily distinguishable from the adjoining tissues by their central position, size, form, and staining reaction. The nucleus is large and the cytoplasm dense and granular. The nucleolus is probably the most conspicuous feature of the nucleus, and appears as a roundish, darkly staining sphere. The chromatin network of the resting nucleus is delicate and finely granular.

In Delicious anthers killed at a later date, the pollen mother cells and the tapetum are distinctly separated (fig. 2). There has been an apparent although perhaps not actual increase in the number of pollen mother cells, and they now number approximately 25 in cross-section. The size of the individual cells, nuclei, and prominent nucleoli also appears larger. The reticulum of the nucleus in these cells is now more granular and distinct, due to the aggregation of the chromatin into more dense threads.

When the pollen mother cells enter synapsis, the chromatin concentrates into an irregular densely staining mass at one side of the nucleus (fig. 3). Occasional threads may be seen protruding from the aggregated mass. Following synapsis the double spireme is formed and the diakinesis stage is initiated.

Heterotypic and homotypic divisions

During the prophase the spireme shortens, thickens, and separates into paired chromosomes, which during diakinesis are distributed around the periphery of the nucleus. Counts of chromosomes in numerous cells at this stage indicate that the haploid number in Delicious is 14. While the apple does not lend itself readily to a study of the double nature of the chromosomes, certain manners of pairing were observed (fig. 11). The paired chromosomes in diakinesis of Delicious are shown in fig. 8, and of Stayman Winesap in fig. 9. In Delicious the spireme seems to shorten and thicken into a definite number of paired chromosomes, which may readily be counted. In Stayman Winesap, however, the chromosomes appear more irregular and variable in size and form. Usually one nucleolus is present in the nucleus during the diakinesis period. It is relatively large, and is generally found toward one end of the somewhat oval nucleus. Occasionally two nucleoli may be seen in the nucleus. When this occurs they appear smaller than when only one is present. The

disappearance of the nucleolus, the dissolution of the nuclear membrane, and the formation of the bipolar spindle terminate the diakinesis stage.

The period between diakinesis and the metaphase of heterotypic division is of extremely short duration. At metaphase the small, somewhat oval chromosomes arrange themselves on the equatorial plate. For a time they lie very close together, but also become separated, and then the pairs may readily be counted. The chromosomes of Delicious always seem to behave regularly, and it is relatively easy to trace them through the various division stages. At metaphase and anaphase the chromosomes lie in slightly different planes. Fourteen haploid chromosomes were counted for Delicious in these stages (figs. 12–15), and checked against the counts made in diakinesis. The fibers of the bipolar spindle extending to the poles are distinct, and terminate in points in the cytoplasm short distances from the plasma membrane. They become less thick in appearance, however, as the close of heterotypic division approaches. Soon after the chromosomes reach the poles, the daughter or dyad nuclei are organized, and preparation is made for the second division.

The fibers of the homotypic division spindles are far less distinct than those of the heterotypic division. After reorganization the dyad nuclei usually divide simultaneously to form the tetrad nuclei. Walls are laid down and the cells spread apart, thus separating the four microspores and allowing the entrance between them of clear, viscid sap.

Pollen grains

At first the microspores are inclosed within the tetrad wall. A period of growth characterized by rounding up of the microspores then occurs. Subsequently the tetrad wall breaks down and the microspores are set free in the loculi of the anther. A short time before the anther is ready to dehisce, the microspore nucleus divides to form the generative and vegetative nuclei of the pollen grain. During and subsequent to this division, the walls of the microspores become greatly thickened and corrugated, until finally the structure of the mature pollen grain results.

Abnormal pollen development

Irregular chromosome behavior in maturation

The regular heterotypic and homotypic divisions of the pollen mother cells previously described are exemplified in Delicious. In Stayman Winesap, on the other hand, irregular chromosome shape, number, and distribution occur. Such irregular chromosome behavior is similar in many respects to that outlined previously in references to the studies in various forms of Rosaceae by Blackburn and Harrison (7), Täckholm (39), Penland (30), Longley (25, 26), and Longley and Darrow (27).

Studies in *Oenothera* are of interest in connection with the occurrence of lagging chromosomes in Stayman Winesap. Rosenberg (32, 33) showed that in hybrids of *Drosera longifolia obovata* with *D. rotundifolia* and *D. longifolia*, some of the smaller chromosomes were left behind in the cytoplasm in the first and second divisions, where they formed small nuclei. In a later work Rosenberg (34) studied a cross of *D. longifolia*, having 40 diploid chromosomes, with *D. rotundifolia*, having 20 diploid chromosomes. At reduction division in the pollen mother cells of this hybrid, 10 bivalent chromosomes and 10 univalents were observed. The bivalents divided normally and traveled to the poles. The univalents, however, passed at random to the poles or remained in the cytoplasm. When they were left in the cytoplasm they proceeded to form other nuclei. The nuclei thus formed were smaller than the ones formed in the usual manner from heterotypic division. In *Oenothera* hybrids Gates (16) observed bodies in the cytoplasm, and concluded that they were chromosomes which had become separated from their fellows on their way to the poles of the spindle. He also found chromosomes in the cytoplasm outside the nuclear wall of tetrad cells, which might or might not be surrounded by a "nuclear membrane." In 21-chromosome offspring of *Oenothera lata*×*O. gigas*, *O. Lamarckiana*×*O. gigas*, and *O. gigas*×*O. Lamarckiana*, Geerts (18) observed the occurrence of 7 pairs of bivalents and 7 univalent chromosomes. In the first division of the former the usual separation of the two members of each of the 7 pairs and their passage to the poles occurred. Geerts found that in the 7 unpaired chromosomes the second

division might be irregular, and some or all of these might fail to reach the pole, and hence would remain outside the daughter nuclei. He further observed that a number of irregular chromosomes or pieces of chromosomes might be distributed to each of the four homotypic poles. In regard to the fate of these fragments of irregular bodies, he stated that when the tetrad nuclei formed, this chromatin material was occasionally taken up by the nucleus, although it usually remained outside the nuclear membrane. Furthermore, he claimed that this chromatin material which remained outside the nucleus often developed into small nuclei in the young pollen grains. In the usual 7 and 8, and in the rare 9 and 6 forms of *O. lata* and *O. semilata*, GATES and THOMAS (**17**) found that the extra chromosomes were frequently left behind, and could be seen fragmenting and degenerating in the cytoplasm.

In *Triticum polonicum*×*T. spelta* hybrids, KIHARA (**22**) noted 14 bivalent and 7 univalent chromosomes in the heterotypic prophase, and that the latter were distributed irregularly at the second mitosis. KIHARA (**23**) further observed lagging univalent chromosomes at heterotypic division in triploid wheat hybrids, which either passed at random to the poles, or remained behind in the cytoplasm and took no further part in nuclear divisions. In cytological studies of wheat crosses, WATKINS (**41**) also noted lagging chromosomes which first appeared during heterotypic division. They were usually less than three in number. During the homotypic division the univalent chromosomes were found to pass to the poles at random, and very often one or more of them failed to reach the poles and were left outside the daughter nuclei in the cytoplasm. SAX (**36**) thought that sterility in *Triticum* hybrids could be explained on the basis of (1) the proportion of univalents to bivalents in the resulting gametes, and (2) the completeness of chromosome sets as determined by both the univalents and bivalents. The former factor accounted for sterility in the F_1, where it was assumed that only those gametes are functional which approach in chromosome numbers the gametes of the parents. The latter factor influenced the F_2 generation, as complete sets were not always found to be present. In the F_2 individuals weak somatic development partially prevented gamete formation, thus adding its effect to sterility due to chromosome combinations.

Heterotypic division

In Stayman Winesap in the diakinesis stage certain deviations from the normal as exemplified in Delicious may be observed. Although good spireme preparations were not plentiful in which to observe any irregularities at this stage, certain features of diakinesis indicate that perhaps not all of the abnormalities of the spireme were due to poor fixation. In diakinesis the chromosomes are arranged around the periphery of the nucleus, although they are irregular in size, shape, and distribution. Some of them appear abnormally large, as though the spireme has failed to fragment completely, and two or more of the bivalents may have remained joined. Others are much smaller than the average and are probably univalents. The univalents often exhibit odd shapes similar to the figures assumed by the chromosomes in late metaphase. In a number of instances the chromosomes, as in fig. 10, appear to be outside the nuclear membrane before the latter structure has completely disappeared. The chromosomes of Stayman Winesap were difficult to count, but in a number of instances, when they were rather evenly distributed throughout the nucleus, counts indicated that at least not all of them were paired and that there were a number of univalents.

During the anaphase and telophase stages of the heterotypic division, the chromosome distribution is extremely irregular in Stayman Winesap. Many of the chromosomes lag on the spindle, and are left outside the daughter nuclei when these organize. Occasionally only one well defined nucleus is formed, and the chromosomes allotted to the other are distributed almost at random throughout the cytoplasm. The lagging chromosomes often organize as miniature nuclei and may persist for some time. Some of the chromosomes lost in the heterotypic division seem to be reincluded in the homotypic division.

Homotypic division

The homotypic division in Stayman Winesap shows considerable irregularity in chromosome behavior. During the interkinesis period fragments of one or more chromosomes, or entire univalents or bivalents, may be observed in the cytoplasm. Figs. 16, 17, and 18 are typical of interkinesis in Stayman Winesap. In fig. 16 the two nuclei are formed, but a large nucleolus is still present in the cytoplasm.

While it may be noted that GATES (16) found nucleoli which took only the orange of the iron-haematoxylin-orange stain, the large one present in this cell stains the intense color typical of chromatin. There is no evidence in the apple, however, that nucleoli are associated with the occurrence of chromosomes in the cytoplasm. In certain cases (figs. 16–18) many chromosomes appear in the cytoplasm during interkinesis. Some are organized as nuclei and others have not progressed so far. Examination of such cells shows that the number of chromosomes in Stayman Winesap at this period exceeds the number for Delicious. It has been pointed out that the chromosomes in Stayman Winesap are not all paired; some are univalents and others bivalents. While counts seemed to indicate more than 28 chromosomes in the dyad of Stayman Winesap, the material at hand was not sufficient to warrant a detailed study of the numbers of bivalents and univalents.

Coarsely knotted, loosely arranged chromosomes resembling spiremes are conspicuous and characteristic of Stayman Winesap (fig. 19). During this time fragmented pieces of chromatin organized into small nuclei appear in the cytoplasm (figs. 20, 25). In the early metaphase stage strands of chromatin may be seen extending from the equatorial plate (figs. 21, 24). During anaphase and telophase chromosomes are found lagging on the spindle, and many of them are left in the cytoplasm when the tetrad nuclei form (figs. 22, 23).

ABNORMALITIES FOLLOWING MATURATION

The extrusion of chromosomes into the cytoplasm during division precedes tetrad and microspore abnormalities. Such abnormalities play a significant rôle in pollen development in Stayman Winesap. Peculiarities in this variety, as contrasted with Delicious, are polyspory or the formation of extra microspores within the tetrad wall, polycary or the presence of additional nuclei in the microspores, and certain types of pollen grain degeneration.

POLYSPORY

Abnormal microspore number within the tetrad wall is known in various plants. A rather extensive list of plants with abnormal microspore number is given by COULTER and CHAMBERLAIN (10).

Among the forms investigated by WILLE (**42**), *Prunus cerasus* is mentioned as containing 5 microspores. Cases in which there were less than the usual number of 4 microspores, namely 2 or 3, were also found by WILLE. BEER (**6**) showed that in *Fuchsia* 6, 8, and even 10 microspores might result from the same pollen mother cell, due to irregular chromosome distribution during the anaphase. In *Rosa*, PENLAND (**30**) observed as high as 10–12 microspores which came from the same pollen mother cell. KIHARA (**23**) found in *Triticum vulgare-secale* hybrids that 2–6 microspores were sometimes formed from a single pollen mother cell.

Explanations of irregular microspore number in *Hemerocallis fulva* were offered by STRASBURGER (**38**), JUEL (**20**), and FULLMER (**15**). The failure of chromosomes to pass to the poles at the first mitosis was believed by STRASBURGER to give rise to small microspores. JUEL agreed with STRASBURGER, and also found that single chromosomes which had become separated from the rest might divide and give rise to nuclei and organize cells. FULLMER attributed the supernumerary microspores in *Hemerocallis fulva* to the division of one or more members of the tetrad. GATES (**16**), however, thought that extra mitosis would not account for such conditions.

More than the usual number of 4 microspores sometimes occur in Stayman Winesap (figs. 32–34). Six microspores have been observed within the tetrad wall. These supernumerary microspores appear normal except in size. Abnormalities of this nature have never been observed in Delicious. While it is possible that one or more of the 4 microspores divide and give rise to additional ones, as found by FULLMER, no evidence of this was observed in the apple. It is more likely that the abnormal numbers observed in Stayman Winesap are a result of irregular meiotic divisions.

It is probable that the chromosomes which appear in the cytoplasm during homotypic division become organized into nuclei and give rise to extra microspores. These upon liberation from the tetrad wall may appear as small pollen grains. Evidence will be presented in a later section to show that small pollen grains are found, and that they occur more frequently in Stayman Winesap than in Delicious.

POLYCARY

Additional nuclei were frequently observed in the microspores of Stayman Winesap, giving rise to the condition known as polycary. Such abnormalities are undoubtedly due to irregular division, as they have never been noticed in Delicious, where regular division is the rule.

DORSEY (12) found in the plum that following heterotypic division some abnormalities appeared in nuclear reorganization. These variations included the formation of as many as three nuclei in the place of one, or rarely the organization of one large and one small nucleus in a single microspore. Sometimes an unusually large nucleus was formed somewhat in advance of the others. Following the liberation from the tetrad wall, the unusually small microspores sometimes found appeared to him to complete the series of variations from the condition noticed in most of the other forms. PENLAND (30) found in *Rosa* that the so-called univalent chromosomes with a separate nuclear membrane might be caught within the wall of a larger microspore.

The two figures to be considered illustrate polycary in Stayman Winesap. In addition to the usual relatively large nucleus in each microspore of fig. 30, there appears in two cases an additional small nucleus, and in the third microspore two smaller nuclei are visible. One of the microspores of fig. 31 shows three miniature chromatin bodies in a small tetrad microspore. Such extra nuclei have never been observed in the mature pollen grains of the apple. The fate of the additional nuclei in the young microspores has not definitely been determined, but failure to observe their presence in the mature pollen grain suggests the possibility that they degenerate.

DEGENERATION OF POLLEN GRAINS

Observation of considerable material indicates that the relative number of normal liberated microspores present in the loculi of anthers of Stayman Winesap is less than in Delicious. This is probably due in part to degeneration of microspores after formation.

Figs. 28 and 29 show tetrads of Stayman Winesap in which nuclei have degenerated in one and two microspores. In such cases, the disorganized chromatin may partially or entirely fill up the por-

tion of the cell formerly occupied by the normal nucleus. When the former condition occurs, the belief that degeneration has taken place seems justified from the evidence. Regarding the latter condition, however, as in fig. 29, such a belief can with difficulty be sustained. The fact that darkly staining masses have sometimes occurred in all four microspores of Delicious, where it was known that the fixation was poor, leads to the opinion that this effect may not be true degeneration. It has been stated previously that Bouin's modified solution has sometimes given poor results at the synapsis stage. It is also probable that the darkly staining masses in two of the microspores (fig. 29) may be due to the killing and fixing solution, or to some difficulty encountered in the many steps through which the material passes in the preparation of permanent mounts. It is difficult to conceive, however, why one or two of the microspore nuclei of a single tetrad should be in such different stages, or be less permeable to the fixative than those which do not show the abnormal nuclear material.

In stages prior to the pollen grain, chromosome behavior in Delicious and Stayman Winesap has been shown to be markedly contrasting. There now remains the matter of associating chromosome behavior with pollen grain abnormalities which are usually accompanied by peculiarities in the cytoplasm, as well as in the chromatin. In contrast to normal development, such abnormalities have to do with the elimination of hereditary material. In other words, factors carried in the chromosomes may be eliminated by suppression in the pollen grain, or they may be carried through this stage.

Dorsey (11) found that sterile pollen in the grape resulted from degeneration processes in the generative nucleus or arrested development previous to mitosis in the microspore nucleus. He showed in the grape that where degeneration took place soon after the division of the microspore nucleus, both the generative and vegetative nuclei might be affected. If the generative cell was well organized before disintegration began, the vegetative nucleus might remain normal. Valleau (40) stated that in the strawberry there was no specific time at which degeneration of the grains within a single anther took place. In most of the sterile forms studied by him, a series of stages

of degeneration appeared from the first period of growth of the microspore to the formation of nearly mature pollen. DORSEY (12) found the earliest evidence of suppression in the plum immediately following the heterotypic division in an extreme hybrid. In other varieties of plum, suppression began after microspore liberation from the tetrad wall. He found suppression taking place at all stages up to maturity of the pollen grain, and was of the opinion that there were no outstanding conditions which would justify placing the beginning of degeneration in the plum earlier than the period of the dyad nuclei, and degeneration at this stage was exceedingly rare. Pollen development in the plum was found by him to proceed through the heterotypic and homotypic divisions with every appearance of being normal. This condition obtained for the most part in the varieties of pure species, as well as in extreme hybrid forms. DORSEY stated that degenerative processes which became so active later did not gain expression as early as this. He believed, however, that the condition found at nuclear reorganization following the heterotypic division justified the conclusion that degenerative processes might begin earlier in the plum than in *Vitis* or *Fragaria*. In the potato, DORSEY (13), BREEZE (8), and YOUNG (45) agreed that in hereditary sterility degeneration was rapid after tetrads had formed and the pollen grains had assumed their characteristic form. BREEZE stated that irregular heterotypic division seemed responsible for some aborted grains in the potato.

It is possible that in some of the forms where degeneration was not observed until dyad reorganization or tetrad formation, initial causes may have occurred previous to the time their effect became apparent. In Stayman Winesap most of the abnormalities associated with degeneration in the nuclei of the pollen grain are initiated during the heterotypic division stages. It is a well recognized fact that as a rule young cells are less highly vacuolated than old ones. In other words, vacuolation is associated to some extent with degeneration. Pollen grains in which the cytoplasm has become markedly vacuolated before complete degeneration of the vegetative or generative nuclei are found in Stayman Winesap. The normal metabolism of cytoplasm is associated with normal development of chromatin. While vacuolation in the pollen grain may not be of as much signifi-

cance as nuclear degeneration, both processes occur more frequently in Stayman Winesap than in Delicious.

The illustrations considered in this paragraph all refer to Stayman Winesap. In fig. 35 the vegetative nucleus has become large and lengthened. The generative nucleus has also extended. Vacuolation has occurred in the cytoplasm. Fig. 36 illustrates an elongated pollen grain in which vacuolation has taken place in the central portion. The vegetative nucleus is large, the nuclear wall thickened, and the plasma contents massed and disorganized. The chromatin in the generative nucleus appears very small. The pollen grain in fig. 37 has become highly vacuolated in one portion, and both nuclei have gathered toward one end. Fig. 39 represents an irregular shaped pollen grain in which vacuolation is evident, and in which the generative nucleus lies in one of the lobes. Fig. 40 shows a pollen grain where the vegetative nucleus and generative nucleus are large. Considerable vacuolation has occurred, and the nuclear contents have become appreciably disorganized. In all these illustrations it is evident that vacuolation has occurred in the pollen grain before complete degeneration of either the vegetative or generative nuclei. Such features are characteristic of one type of abnormality found in the pollen grains of Stayman Winesap. On the other hand, nuclear degeneration may begin in the pollen grain of Stayman Winesap before the cytoplasm becomes excessively vacuolated. In fig. 41 a pollen grain of Stayman Winesap is shown in which the cytoplasm appears relatively normal, but the generative nucleus has degenerated. Degeneration of this nature may occur in either one of the nuclei, or in both. It may occur at different times in the two nuclei, or simultaneously. Such degeneration seems to be associated with irregular chromosome behavior in previous stages.

Variability in size of pollen grains of different varieties

A preliminary examination of the mature pollen of Delicious and Stayman Winesap indicated that there were marked differences in size and shape between the grains of the two varieties. Size measurements were then made of pollen grains of these varieties and Paragon, which is a member of the Winesap group. In table III is given the frequency distribution, based on diameter measurements of 1000 pollen grains of Delicious, Paragon, and Stayman Winesap.

The frequency data indicate that the mean is much lower in Stayman Winesap and Paragon than in Delicious. BEAUMONT and KNIGHT (5) found that abnormally large and small grains failed to germinate. It will be noted that there are relatively few of these types in Delicious, and that the pollen of this variety is distinctive for the large number of medium sized grains. On the contrary, in Paragon and Stayman Winesap the number of medium sized grains is decidedly smaller. These two varieties are characterized by a great number of small grains and a number of large ones. In regard to the size of pollen grains, significance may perhaps be attached to the works of SAX (35) and KIHARA (23) on the relation of chromosome number to the size of the pollen grain. They believed that the size of the pollen grain of wheat depended on the chromosome number; the greater the number the greater the size. KIHARA also found that dwarf pollen grains are often produced from lagging chromosomes which failed to reach the poles during homotypic division. These small pollen grains were incapable of germination. Large pollen grains which resulted from failure of homotypic division to take place were also observed by him. The reason for the relatively greater frequency of large pollen grains in Stayman Winesap and Paragon than in Delicious has not been determined definitely. It seems probable, however, that the abnormally large pollen grains found in Stayman Winesap and Paragon, like the small ones, are a result of irregular chromosome distribution during meiotic divisions.

TABLE III

FREQUENCY DISTRIBUTION BASED ON DIAMETER MEASUREMENTS OF 1000 POLLEN GRAINS OF DELICIOUS, PARAGON, AND STAYMAN WINESAP

VARIETY	CLASS SIZE OF POLLEN GRAINS IN MICRONS									
	14.9	19.9	24.9	29.9	34.9	39.9	44.9	49.9	54.9	59.9
Delicious	.....	1	6	15	10	100	621	229	18	.....
Paragon	.....	8	29	71	119	242	330	159	37	5
Stayman Winesap	6	8	12	104	172	115	273	241	61	8

Conclusions

The following conditions were thought to exist in the Winesap group of apple varieties when the present investigation was under-

taken: (1) there was almost complete self and inter-sterility; (2) varieties of the group when used as pollen parents in controlled crossing had consistently given a low set of fruit; (3) pollen germination of the members was low; and (4) non-viability was associated in some degree with size of pollen grain.

From a study of a member of the Winesap group, Stayman Winesap, it is found that the chromosomes in the pollen mother cells occur as bivalents and univalents, and that they are paired and distributed irregularly. During meiotic divisions some of the chromosomes lag on the spindle, are extruded into the cytoplasm where they may become organized as small nuclei, and are left outside the daughter nuclei when these form. This irregular behavior of the chromosomes leads to extra microspores and additional nuclei in some microspores, is reflected in abnormal size and shape of the pollen grains, and results in a high degree of pollen sterility. Thus in Stayman Winesap pollen sterility, irregular chromosome behavior, polyspory, and polycary exist. Such conditions are considered to be associated with hybrid forms.

Summary

1. Pollen development in Delicious is normal, while in Stayman Winesap it is characterized by certain abnormalities.

2. Instead of the 14 pairs of chromosomes found in Delicious, there are a number of bivalents and univalents in Stayman Winesap which are irregularly arranged at diakinesis.

3. During heterotypic division in Stayman Winesap some of the chromosomes lag on the spindle and are left in the cytoplasm when the daughter nuclei form.

4. Counts were made which indicate that more than 28 chromosomes are present in the dyad cell of Stayman Winesap.

5. The coarsely knotted, loosely arranged chromosomes resembling spiremes with chromatin extending from the equatorial plate at early metaphase are characteristic of Stayman Winesap during homotypic division. Sometimes only one well defined daughter nucleus seems to be formed. Chromosomes also appear in the cytoplasm during the second division and become organized into small nuclei.

6. In Stayman Winesap more than the usual number of four microspores are frequently found within the tetrad wall (polyspory).

7. Additional small nuclei are sometimes observed in Stayman Winesap in the microspores (polycary); these have never been seen in the later stage of the mature pollen grain.

8. The relative number of normal microspores in the loculi of the anthers is less in Stayman Winesap than in Delicious; thus there is a reduction in the quantity of pollen produced.

9. Degeneration of the pollen grains occurs more frequently in Stayman Winesap than in Delicious. Vacuolation occurs in the cytoplasm of the pollen grain before complete degeneration of the nuclei; or, on the other hand, the nuclei are affected while the cytoplasm is relatively normal. The latter process, which is probably more significant than the former, seems to be associated with irregular chromosome behavior in previous stages.

10. Abnormally small and large pollen grains arise as a result of irregular meiotic divisions.

11. Measurements and grouping of pollen grains indicate that Paragon and Stayman Winesap differ from Delicious in the greater frequency with which small and large pollen grains occur, and in the relative decrease of medium sized grains.

The work was begun at the suggestion of J. H. BEAUMONT, Assistant Horticulturist, Minnesota Agricultural Experiment Station. The writer wishes to extend his gratitude to Dr. BEAUMONT for his advice and help in planning and carrying out the investigation, and to acknowledge his indebtedness to Professor W. H. ALDERMAN, Chief of the Horticultural Division, University of Minnesota, for encouragement in the study.

OHIO AGRICULTURAL EXPERIMENT STATION
WOOSTER, OHIO

LITERATURE CITED

1. ALLEN, C. E., Studies on cell division in the albino rat. Anat. Rec. **10**:565–584. 1916.
2. AUCHTER, E. C., Apple pollen and pollination studies in Maryland. Proc. Amer. Soc. Hort. Sci. 51–80. 1921.

3. AUCHTER, E. C., Importance of proper pollination in fruit yields. Peninsula (Md. and Del.) Hort. Soc. November 1923.
4. BALLARD, W. R., Methods and problems in pear and apple breeding. Maryland Agric. Exp. Sta. Bull. 196. 1916.
5. BEAUMONT, J. H., and KNIGHT, L. I., Apple pollen germination studies. Proc. Amer. Soc. Hort. Sci. 151–163. 1922.
6. BEER, R., The supernumerary pollen grains of *Fuchsia*. Ann. Botany **21**: 305–307. 1907.
7. BLACKBURN, K. B., and HARRISON, J. W., The status of the British rose forms as determined by their cytological behavior. Ann. Botany **34**:159–187. 1921.
8. BREEZE, J. S. G., Degeneration in anthers of potato. Gard. Chron. **70**: 274–275. 1921.
9. CLOSE, C. P., Pollination of apples. 14th Ann. Rept. Delaware Agric. Exp. Sta. 1902.
10. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of angiosperms. New York. 1909.
11. DORSEY, M. J., Pollen development in the grape with special reference to sterility. Minn. Agric. Coll. Sta. Bull. 144. 1914.
12. ———, A study of sterility in the plum. Genetics **4**:417–488. 1919.
13. – ——, A note on the dropping of flowers in the potato. Jour. Heredity **10**:226–228. 1919.
14. ———, The set of fruit in apple crosses. Proc. Amer. Soc. Hort. Sci. 82–94. 1921.
15. FULLMER, E. L., The development of the microsporangia and microspores of *Hemerocallis fulva*. BOT. GAZ. **28**:81–88. 1899.
16. GATES, R. R., Pollen development in hybrid of *Oenothera lata*×*O. Lamarckiana*, and its relation to mutation. BOT. GAZ. **43**:81–115. 1907.
17. GATES, R. R., and THOMAS, N., A cytological study of *Oenothera* mut. *lata* and *Oe.* mut. *semilata* in relation to mutation. Quart. Jour. Micr. Sci. **59**:523–571. 1914.
18. GEERTS, J. M., Cytologische Untersuchungen einiger Bastarde von *Oenothera gigas*. Ber. Deutsch. Bot. Gesells. **29**:160–166. 1911.
19. GOWEN, J. W., Self-sterility and cross-sterility in the apple. Maine Agric. Exp. Sta. Bull. 287. 1920.
20. JUEL, H. O., Die Kerntheilungen in den Pollenmutterzellen von *Hemerocallis fulva* und die bei denselben auftretenden Unrelegmassigkeiten. Jahrb. Wiss. Bot. **30**:205–226. 1897.
21. KEIL, J. B., Apple pollinations. Monthly Bull. Ohio Agric. Exp. Sta. **8**:51–58. 1923.
22. KIHARA, H., Über cytologische Studien bei eiginen Getreidearten. I. Bot. Mag. Tokyo **32**:17–38. 1919.
23. ———, Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die

Sterilitat in den Bastarden. Memoirs Coll. Sci. Kyoto Univ. **1**: pp. 202. 1924.

24. KNOWLTON, H. E., Pollen abortion in the peach. Proc. Amer. Soc. Hort. Sci. 67–69. 1924.
25. LONGLEY, A. E., Cytological studies in the genus *Rubus*. Amer. Jour. Bot. **11**:249–282. 1924.
26. ———, Cytological studies in the genus *Crataegus*. Amer. Jour. Bot. **11**: 295–317. 1924.
27. LONGLEY, A. E., and DARROW, G. M., Cytological studies of diploid and polyploid forms in raspberries. Jour. Agric. Res. **27**:737–748. 1924.
28. MEYER, J. VON, Die Crataegomespili von Bronvrux. Zeitschr. Ind. Aest. Vererbungslehre **13**:293–333. 1915.
29. MORRIS, O. M., Studies in apple pollination. Wash. Agric. Exp. Sta. Bull. 163. 1921.
30. PENLAND, C. W. T., Cytological behavior in *Rosa*. BOT. GAZ. **76**:403–410. 1923.
31. POWELL, G. H., Pollination of pears and apples. 12th and 13th Ann. Rept. Dela. Agric. Exp. Sta. 1900–1901.
32. ROSENBERG, O., Das Verhalten der Chromosomen in einer hybriden Pflanze. Ber. Deutsch. Bot. Gesells. **21**:110–119. 1903.
33. ———, Über die Tetradentheilung eines *Drosera* Bastardes. Ber. Deutsch Bot. Gesells. **22**:47–53. 1904.
34. ———, Cytologische und morphologische Studien an *Drosera longifolia*× *rotundifolia*. K. Svensk. Vetensk. Akad. Handl. **43**:1–64. 1909.
35. SAX, K., Sterility in wheat hybrids. II. Chromosome behavior in partially sterile hybrids. Genetics **7**:513–552. 1922.
36. ———, The relation between chromosome number, morphological characters, and rust resistance in segregates of partially sterile wheat hybrids. Genetics **8**:301–321. 1923.
37. STRASBURGER, E., Über den Theilungsvorgang der Zellkerne und das Verhaltnis der Kerntheilung. Archiv. Mikr. Anan. **21**:476–490. 1882.
38. TÄCKHOLM, G., On the cytology of the genus *Rosa*. A preliminary note. Svensk. Bot. Tidskr. **14**:500–311. 1920.
39. ———, Zytologische Studien über die Gattung *Rosa*. Acta Horti. Bergiana **7**:97–381. 1922.
40. VALLEAU, W. D., Sterility in the strawberry. Jour. Agric. Res. **12**:613–669. 1918.
41. WATKINS, A. E., Genetic and cytological studies in wheat. Jour. Genetics **14**:129–171. 1924.
42. WILLE, N., Über die Entwickelungsgeschichte der Pollenkorner der Angiospermen und das Wachtshum der Membranen durch Intussusception. Christiana. 1886.
43. WILLIAMS, C. G., 42d Ann. Rept. Ohio Agric. Exp. Sta. Bull. 373. 1923.
44. WILSON, E. B., The cell in development and heredity. New York. 1925.

45. YOUNG, W. J., The formation and degeneration of germ cells in the potato. Amer. Jour. Bot. 10:325–335. 1922.

EXPLANATION OF PLATES XII–XIV

PLATE XII

FIG. 1.—Delicious: relatively early stage of pollen mother cells.

FIG. 2.—Delicious: later pollen mother cells; tapetum separated from pollen mother cells, and middle layers beginning to break down.

FIG. 3.—Delicious: anther tissues when pollen mother cells are in synapsis.

FIG. 4.—Delicious: degeneration of tapetal cells at time of tetrad formation.

FIG. 5.—*Pyrus ioensis* Bailey: degeneration of tapetal cells during homotypic division.

FIG. 6.—Delicious: tapetal cells losing their identity; middle layers elongated in different directions from previous stages; pollen grain stage.

FIG. 7.—Delicious: pollen grain stage; tapetum practically disappeared.

PLATE XIII

FIG. 8.—Delicious: diakinesis.

FIG. 9.—Stayman Winesap: diakinesis; irregular arrangement of chromosomes.

FIG. 10.—Stayman Winesap: diakinesis; chromosomes outside nucleus in cytoplasm.

FIG. 11.—Stayman Winesap: certain manners of pairing of chromosomes.

FIGS. 12, 13.—Delicious: 14 paired chromosomes at metaphase.

FIG. 14.—Delicious: heterotypic anaphase.

FIG. 15.—Delicious: late heterotypic anaphase.

FIG. 16.—Stayman Winesap: interkinesis; large nucleolus and chromosomes in small nuclei in cytoplasm.

FIG. 17.—Stayman Winesap: interkinesis; only one well defined daughter cell formed; chromosomes and nuclei in cytoplasm.

FIG. 18.—Stayman Winesap: interkinesis; bivalents and univalents in cytoplasm; number of chromatin bodies present exceeds number in Delicious.

FIG. 19.—Stayman Winesap: knotted, loosely arranged chromosomes in one nucleus resemble a spireme, and are at early metaphase in other nucleus.

FIG. 20.—Stayman Winesap: homotypic spireme appearance; chromosomes organized into smal nuclei in cytoplasm.

FIG. 21.—Stayman Winesap: homotypic metaphase; strand of chromatin extending from equatorial plate; chromatin in cytoplasm.

FIG. 22.—Stayman Winesap: homotypic telophase; nuclei in cytoplasm.

FIG. 23.—Stayman Winesap: homotypic telophase; large number of nuclei in cytoplasm.

FIG. 24.—Stayman Winesap: homotypic division; metaphase; chromatin seen extending from equatorial plate.

FIG. 25.—Stayman Winesap: dyad reorganization; chromosomes at side of reforming nuclei.

FIG. 26.—Stayman Winesap: homotypic division; chromosomes in cytoplasm.

PLATE XIV

FIG. 27.—Delicious: normal tetrad; only 3 microspores shown.

FIG. 28.—Stayman Winesap: chromatin disorganization in one microspore; additional miniature nucleus in another.

FIG. 29.—Stayman Winesap: disorganization of nuclei in two microspores of a tetrad, probably due to poor fixation.

FIG. 30.—Stayman Winesap: additional nuclei (polycary) in microspores.

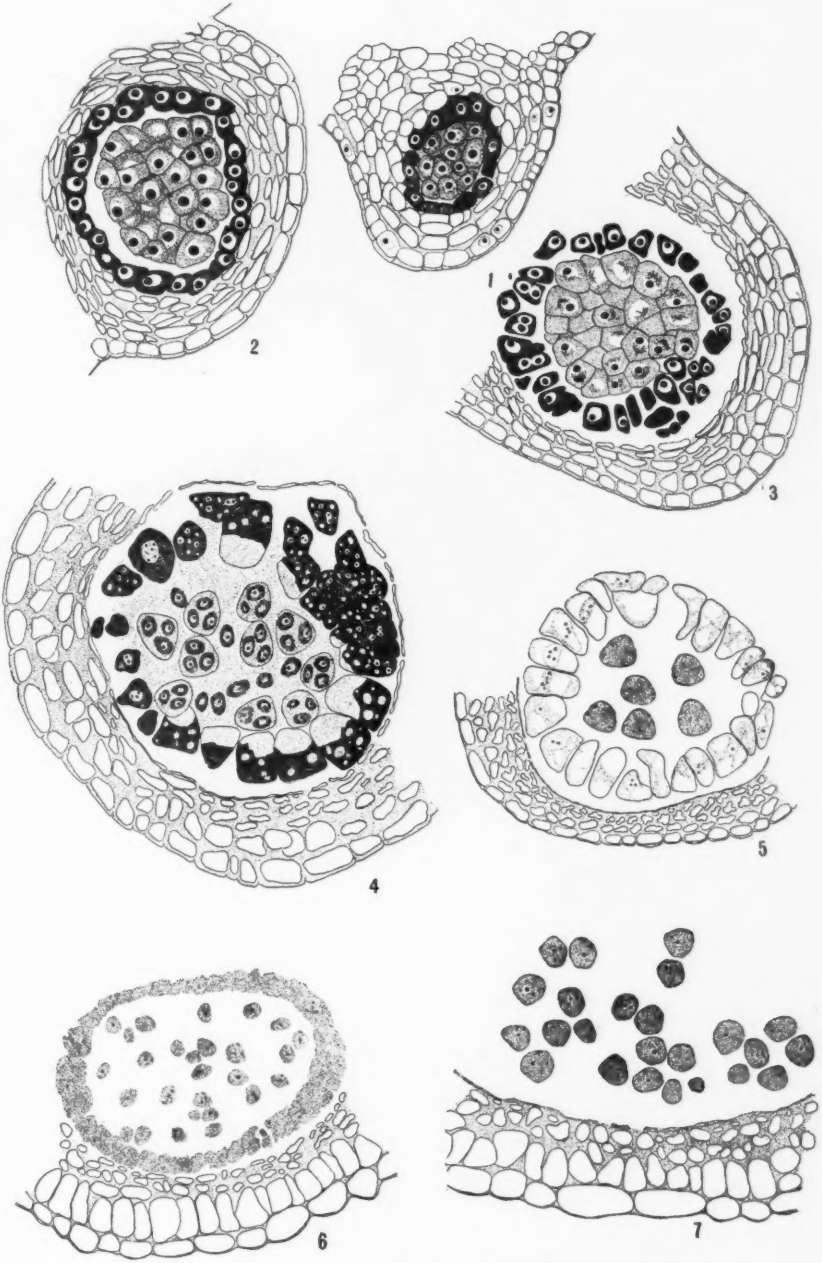
FIG. 31.—Stayman Winesap: three miniature nuclei in a microspore.

FIGS. 32–34.—Stayman Winesap: more than usual number of microspores within tetrad wall (polyspory).

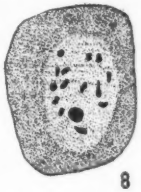
FIGS. 35–40.—Stayman Winesap: nuclear disorganization associated with vacuolation of cytoplasm.

FIG. 38.—Delicious: normal pollen grain.

FIG. 41.—Stayman Winesap: degeneration in generative nucleus of pollen grain before cytoplasm becomes excessively vacuolated.



SHOEMAKER on POLLEN DEVELOPMENT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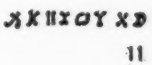
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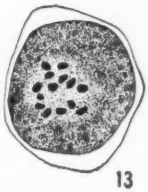
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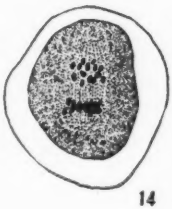
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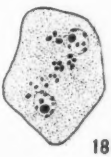
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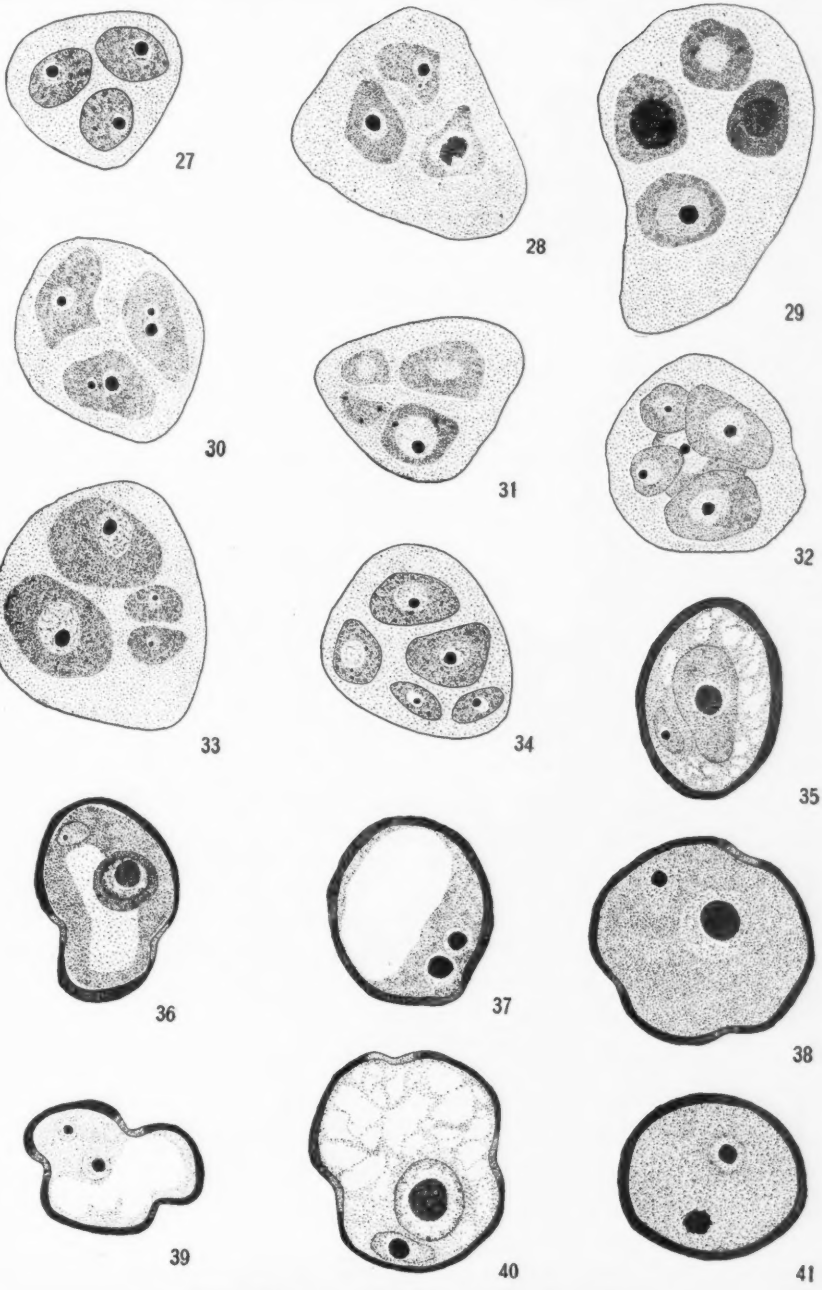


SHOEMAKER on POLLEN DEVELOPMENT

The papers by PFEIFFER, REID, ECKERSON, and PURDY in this issue are being published upon their recent receipt at the expense of the Boyce Thompson Institute for Plant Research.

MICROCHEMICAL AND MORPHOLOGICAL STUDIES OF EFFECT OF LIGHT ON PLANTS

NORMA E. PFEIFFER

(WITH PLATE XV AND FOUR FIGURES)

Microchemical studies

In recent years there has developed a tendency to correlate with macrochemical analyses as thorough microchemical studies of the fresh tissues as the investigator can make. By the combination of the two methods, the quantitative results obtained by the one can in some degree be checked, and helpful information as to the distribution and localization of the substances involved can be contributed by the other.

There has been slight evidence of this tendency in investigations dealing with the effect of light duration on plants. The earliest work in which artificial illumination was used (MANGON **19**, PRILLEUX **23**) served to establish production and activity of chlorophyll as in sunlight. There followed a number of investigations in which artificial light of one sort or another, in varying intensities, was used to supplement daylight. In the major portion of this work, possibly because of commercial importance, much attention was given to the effect on the form, size, weight, time of flowering, and seed production of the plant (ADAMS **1, 2, 3**, BAILEY **5**, CORBETT **7**, DEHÉRAIN **9**, HARVEY **15**, OAKLEY and WESTOVER **22**, SIEMENS **24**, TIEDJENS **25**, WANN **26**, WANSER **27**). In relatively few investigations (GARNER and ALLARD **12, 13**, LUBIMENKO and SŽEGLOFF **18**, NIGHTINGALE **21**) the chemical background for these external changes was touched upon. TIEDJENS (**25**) briefly tested for carbohydrates, and NIGHTINGALE (**21**) especially supported his macroanalyses with microchemical data.

It seemed important for more complete knowledge of the situation to apply both macrochemical (ARTHUR **4**) and microchemical methods to the situations occurring in plants grown in different daily periods.

CONDITIONS OF PRESENT EXPERIMENT

The light conditions varied in intensity and amount in the different sets. In one room plants were grown in artificial light supplied by twenty-five 1500-watt lamps, with an intensity varying from 780 foot candles at the beginning of the run to 352 foot candles at the end, as measured by the Macbeth Illuminometer.[1] As determined by the pyrheliometer, the measurement at the beginning was 2.406 gm. calories per sq. cm. per minute, and 1.938 gm. calories at the end. The daily periods of illumination were for 5, 7, 12, 17, 19, and 24 hours for the different sets of plants in this room. There was a fluctuating supply of carbon dioxide, averaging 0.3 per cent. The temperature was constant at 25.5° C., and the humidity at 80 per cent of saturation.

Plants in two houses equipped with a gantry crane[2] carrying forty-eight 1000-watt lamps, had 6 hours of additional illumination beyond natural daylight. In one case this artificial light was supplied from 6:00 P.M. to midnight, in the other from midnight to 6:00 A.M. The former had an extra supply of carbon dioxide, the latter the usual atmosphere. In both cases the artificial light was 383.69 foot candles (Macbeth Illuminometer) and 0.465 gm. calories per sq. cm. per minute (pyrheliometer) at the beginning of the experiment, and gradually diminished to 141.5 foot candles (Macbeth Illuminometer) and 0.323 gm. calories (pyrheliometer) at the end. A house with ordinary atmosphere and the natural light conditions of the season (March, April, and beginning of May) served for the control plants. The temperature and humidity were as in the continuous light room.

METHODS

From previous experience it was deemed that an intensive study of the tomato (*Lycopersicum esculentum*), which shows a decided reaction to longer periods of light, would be of importance. Studies were made of individual plants of the variety Bonny Best in each of the series in different daily periods of illumination, at various in-

[1] Data supplied by J. M. ARTHUR.

[2] Greenhouses equipped with gantry crane are described and illustrated in Contr. Boyce Thompson Inst. Plant Research 1:17. figs. 5, 6. 1925.

TABLE I

TOMATO; MICROCHEMICAL ANALYSES

HOURS LIGHT	AGE (DAYS)	SUGARS	STARCH	BIURET	NITRATES	PHOSPHATES	MAGNESIUM	GENERAL RATIO
5	7	Very little	Very little	Good	Very high	.....	.....	Low carbohydrates: low protein
	15	Very little	Very little	Little	Much	.....	.....	
	25	Very little	Very little	Little	Excessive	.....	.....	
	43	Increase	Very little	Little	Excessive	Negative	Negative	
7	7	Very little	Very little	Little	Very high	.....	.....	Low carbohydrates: low protein
	16	Little	Little	Little	Much	.....	.....	
	28	Little	Little	Little	Excessive	.....	.....	
	44	Little	Little	Little	Excessive	Very little	Negative	
12	6	Little	Moderate	Little+	Very high	.....	.....	Slightly increased carbohydrates: slightly increased protein
	16	Little	Decrease	Little+	Much	.....	.....	
	25	Little	Decrease	Negative	Excessive	.....	.....	
	44	Increase	Little	Little	Moderate	Negative	Negative or trace	
17	8	Little	Little	Moderate	Very much	.....	.....	Higher carbohydrates: protein as in 12 hours
	16	Little	Little	Moderate	Much	.....	.....	
	24	Increase	Increase	Little	Excessive	.....	.....	
	38	Greater increase	Increase	Little	Moderate or negative	Very little	Trace to fair	
19	8	Little	Little	Moderate	Very much	.....	.....	Low carbohydrates: low protein
	16	Slight increase	Little	Little	Much	.....	.....	
	24	Same	Little	Little	Excessive	.....	.....	
	37	Increase	Slight increase	Decrease	Excessive	Negative or little	Little to much	
24	9	Little	Little	Moderate	Excessive	.....	.....	Slightly increased carbohydrates: higher protein
	17	Little	Little	Little+	Very much	.....	.....	
	25	Little	Little	Little+	Excessive	.....	.....	
	48	Great increase	Moderate	Little to good	Negative	Negative or little	Fair	
Control	7	Negative	Little	Good	Excessive	.....	.....	Moderate carbohydrates: lower protein
	18	Very little	Fair+	Fair	Excessive	.....	.....	
	27	Increase	Fair+	Little	Excessive	.....	.....	
	45	Same	Moderate	Decrease	Negative	Negative or trace	Moderate	
Gantry crane house, $+CO_2$	7	Very little	Very little	Little+	Excessive	.....	.....	Moderate carbohydrates: low protein
	16	Fair	Fair	Little+	Excessive	.....	.....	
	35	Moderate	Moderate	Little+	Negative	Fair	Moderate	
Gantry crane house, usual atmosphere	8	Negative	Little	Fair	Excessive	.....	.....	Moderate carbohydrates: less protein
	16	Fair	Little+	Little	Excessive	.....	.....	
	35	Moderate	Fair	Very little to little	Trace	Negative	Much	

tervals, until the plants were about 6 weeks old. Analyses were made at as near the end of the daily exposures to light as possible. Tests were made for the carbohydrates, protein, nitrates, magnesium, calcium, and phosphorus, on free-hand sections in each of 5 regions of the plant, namely, root, lower stem, middle stem, upper stem, and leaf, with the object of obtaining an idea of the plant chemistry in different regions. Aside from this intensive study of a single form, buckwheat (*Fagopyrum esculentum*) of Henderson's Japanese variety was considered, with a longer interval between analyses, and a few other forms were examined for supplementary data at but one time. Unfortunately, in this phase of the work there is some error due to individual differences, since there were not enough plants of a single kind to avoid this difficulty.

Standard tests were used, Flückiger's reaction for fructose, glucose, and dextrin; potassium iodide solution of iodine for starch; a similar solution and biuret test for proteins; diphenylamine in 75 per cent sulphuric acid for nitrates; formation of ammonium magnesium phosphate crystals for magnesium and phosphorus, by use of appropriate reagents in each case; and 5 per cent sulphuric acid for calcium.

Results

In the case of tomato, the results of tests, made at four intervals until the plants were about 6 weeks old, can be shown best in tabular form (table I), giving the general conclusion as to the content of the plant from a study of all the regions. This table does not take into account the amount of tissue development, which is more properly considered under the anatomical discussion. Similar tables bring out the important points in regard to buckwheat (table II), as determined at two stages, when the plants were about two and a half weeks old and 7 weeks old. In four o'clock (*Mirabilis Jalapa*) (table III) the aerial part only was tested when the plants were about 5 weeks old. At the conclusion of the experiment, when about 10 weeks old, the roots were examined. The table dealing with the earlier tests shows the part of the body commonly used for gross analyses; the latter, dealing with the organ commonly overlooked, gives interesting data in regard to reserves in different light durations (see also morphology). Observations on other plants, although too scat-

TABLE II

BUCKWHEAT; MICROCHEMICAL ANALYSES

HOURS LIGHT	APPROXIMATE AGE (DAYS)	SUGARS	STARCH	BIURET	NITRATES	PHOSPHATES	MAGNESIUM	GENERAL RATIO
5	17	Little	Little	Fair	Much	.....	.....	Low carbohydrates: low protein
	48	Decrease	Increase	Little	Excessive	Negative	Trace	
7	17	Little	Moderate	Little+	Much	.....	.....	Low carbohydrates: low protein
	48	Decrease	Increase	Decrease	Excessive	Excessive	Excessive	
12	17	Little	Fair	Moderate	Very much	.....	.....	Low carbohydrates: low protein
	48	Decrease	Increase	Little	Excessive	Much	Excessive	
17	17	Little	Moderate	Fair	Much	.....	.....	High carbohydrates: low protein
	48	Increase	Increase	Little	Negative	Trace	Little	
19	17	Little+	Moderate+	Fair	Much	.....	.....	High carbohydrates: low protein
	48	Increase	Increase	Little−	Negative	Moderate	Much	
24	17	Little+	Little+	Fair	Much	.....	.....	High carbohydrates: more protein (than 17 or 19 hours)
	48	Slight increase	Good increase	Fair	Trace	Fair	Very much	
Control	17	Little+	Little+	Moderate	Much	.....	.....	Low carbohydrates: low protein
	48	Decrease	Decrease	Little	Excessive	Much	Moderate+	
Gantry crane house, $+CO_2$	17	Little+	Fair	Fair	Much	.....	.....	High carbohydrates: low protein
	48	Increase	Increase	Decrease	Trace	Much	Little	
Gantry crane house, usual atmosphere	17	Little−	Fair	Fair	Much	.....	.....	High carbohydrates: fair protein
	48	Very slight increase	Slight decrease	Slight decrease	Fair	Fair	Fair+	

TABLE III

FOUR O'CLOCK; MICROCHEMICAL ANALYSES

HOURS LIGHT	APPROXIMATE AGE (WEEKS)	SUGARS	STARCH	BIURET	NITRATES	PHOSPHATES	MAGNESIUM	GENERAL RATIO
	Aerial parts							
5	5	Very little	Little	Much	Excessive	Little	Little	Low carbohydrates: high protein
7	5	Little	Little	Much	Very much	Very little	Moderate	Low carbohydrates: high protein
12	5	Slight increase	Slight increase	Much	Excessive	Negative	Much	Low carbohydrates: high protein
17	5	As above	As above	Slight increase	Excessive	Much	Very much	Slightly more carbohydrates: high protein
19	5	Fair	Little	Moderate	Excessive	Much	Very much	Slightly more carbohydrates: high protein
24	5	Moderate	Little	Fair	Excessive	Negative	Moderate	Higher carbohydrates: low protein
Control	5	Very little	Little	Moderate+	Excessive	Very little	Moderate	Low carbohydrates: high protein
Gantry crane house, $+CO_2$	5	Fair	Fair+	Moderate+	Excessive	Negative	Moderate+	Higher carbohydrates: high protein
Gantry crane house, usual atmosphere	5	Moderate	Moderate	Moderate+	Excessive	Negative	Moderate	Higher carbohydrates: high protein
	Roots							
5	10	Negative	Moderate	Fair	Excessive	Negative	Negative	Low carbohydrates: low protein
7	10	Negative	Much	Fair	Excessive	Negative	Negative	Low carbohydrates: low protein
12	10	Very little	Much	Fair−	Excessive	Trace	Negative	Higher carbohydrates: low protein
17	10	Very little	Very much	Fair	Fair	Moderate	Fair	High carbohydrates: low protein
19	10	Little	Excessive	Fair	Fair	Fair	Little	High carbohydrates: low protein
24	10	Trace	Excessive	Fair	Little	Much	Much	High carbohydrates: low protein
Control	10	Negative	Much	Fair−	Very much	Fair	Much	Less high carbohydrates: low protein
Gantry crane house, $+CO_2$	10	Very little	Excessive	Fair+	Fair−	Much	Much	High carbohydrates: low protein
Gantry crane house, usual atmosphere	10	Little	Excessive	Fair−	Very much	Fair	Much	High carbohydrates: low protein

tered to be worthy of tabulation, are depended upon in the following discussion.

Discussion

In coordinating the facts observed, one finds that usually in the short exposures to light there is a low carbohydrate and low protein content, which is coupled, as may be seen in the anatomical work, with relatively less tissue production than in longer exposures. The most marked exception to this ratio is seen in the four o'clock, where the aerial parts early show a relatively higher protein content. In the longer exposures, under similar atmospheric conditions, there is evident a tendency toward greater production of carbohydrates without a proportionately increased ability to utilize the manufactured product in protein formation and tissue development. In tomato and four o'clock this increase, evident in the 12-hour exposure, is even clearer in 17 hours of light. The tomato suffers injury, however, in this and higher durations, with the result that photosynthetic ability falls markedly, so that in 19 hours the carbohydrates are again relatively low. A comparable fall does not appear in the buckwheat, which seems better able to utilize longer exposures. In all cases, except possibly four o'clock, there is a greater carbohydrate content in the plants in the gantry crane house with extra carbon dioxide than in those with the ordinary atmosphere.

In comparisons of the nitrates present in the different sets of conditions in different plants, variation is evident at once. The tomato in longer exposures, as in shorter, shows great amounts of nitrate present. Only in the control and in the two houses receiving extra light beyond daylight are there negative results or only traces. In four o'clock, on the other hand, there are ample nitrates in the aerial part in all series, although the subterranean parts show much less at the end of the run in 17, 19, and especially in 24 hours' exposures. Practically the same thing is true in buckwheat as a whole as in four o'clock roots. In other forms examined, it was found that usually the nitrates ran lowest in the gantry house with extra carbon dioxide. There is a possibility that the plants in this series, making the great growth that they do, draw too heavily on the soil, and may not have adequate available nitrates at the end of the run. This point will be guarded more carefully in further work. It may be

pointed out, however, that such low nitrate content in these plants was found at various stages in several plants, and so was not usually due to low soil supply.

Data in regard to the occurrence of phosphates and magnesium are as yet inadequate for drawing general conclusions. Both appear relatively lower as a rule in the shorter exposures, due probably to proportionately greater utilization in production of proteins and tissue formation.

Anatomical studies

Literature

The most comprehensive study of the anatomical differences brought about by artificial light was made by Bonnier (**6**). Using arc lights as continuous light or for periods of 6 hours in the morning and 6 hours in the afternoon, with fairly constant temperature and humidity, he grew a wide variety of forms and made studies of stem and leaf characters. He drew the following conclusions in regard to the effect of continuous light: (1) the chlorophyll is greater in amount and more uniformly distributed in all the cells containing it in the normal plant; (2) the leaf blade structure is simplified, that is, the palisade is less distinct or entirely lacking and the epidermis has thinner walls; (3) the stem structure is simplified, with late or poorly developed cork, less distinct endodermis, less lignification and sclerification. He also found that the structures in discontinuous electric light were more like those in discontinuous solar light than those in continuous electric illumination. He thinks of continuous light as producing a result much like etiolation except for greater greenness.

Lubimenko and Sžegloff (**18**), dealing with periods of light from 14 hours down to 4, found greater concentration of chlorophyll in the longer exposure, by spectrocolorometric methods rather than by chloroplast study.

More recently, Massart (**20**) studied the effect of continuous light on leaf structure. With a range of forms including representatives from Hepaticae, *Selaginella*, and flowering plants, he used intervals of 6, 12, 18, and 24 hours' exposure to light. He comes to the conclusion that effects on form and structure are more dependent on

intensity than on duration, and that in the intervals used, continuous and discontinuous light affect assimilatory organs in the same way.

Miss DEATS (8), in her anatomical studies of pepper and tomato grown in intervals of 6.5 hours (short day), 17.5 hours (long day), and the normal day period, found that the amounts of bast and xylem in stems varied directly with the length of day. There was variation in the thickness of their cell walls in the same direction. Similarly the size of the epidermal cells and the amount of cork varied directly with the duration of light. In leaves, the greatest thickness and size and depth of greenness occurred in long day plants, the least in short day.

RESULTS

STEM STRUCTURE.—An attempt was made to follow the degree of development of the stem whenever sections were made for microchemical studies. Inasmuch as only a single specimen was used each time, there is bound to be individual variation in the results. The plants were not uniform in development, and one must be aware of this fact.

It seemed that the structure near the base of the stem is the most conservative that one could use for demonstration of results, since the region used could be more exactly determined as a definite distance from soil surface. "Middle stem" is less exact, since plants attained different heights in different light intervals, and the "middle" would be at various levels. Similarly the tip of the stem and the roots, which varied from plant to plant, seem to add less to our knowledge of how the light affected the plant than the base of the stem. I therefore made diagrams of this region in tomato (text figs. 1, 2) and buckwheat (text fig. 3), to show the relative amounts of tissue developed in the different sets of conditions. In tomato these correspond roughly to each other in the different series, sometimes with variation of several days between comparable examinations. This variation and individual variation must both be considered in appreciating how well such diagrams portray the actual effect. Text figs. 1 and 2 show the stems at the time of the first, third, and fourth microchemical analyses (compare table I) in all series up to 24 hours' illumination; of the first, second, and third in the gantry crane

houses; and first, second, and fourth in the control set. It should be borne in mind, therefore, that the second diagram of the control shows a younger plant (7 days' difference) than most of the series,

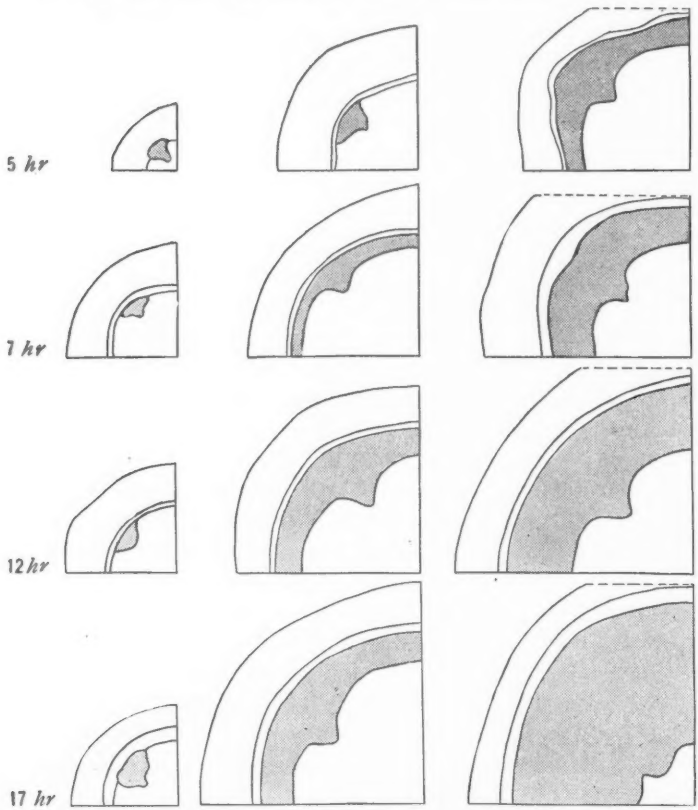


FIG. 1.—Diagrams of bases of stems of tomatoes grown in daily light durations of 5, 7, 12, and 17 hours, showing proportions of tissues at time of first, third, and fourth microchemical analyses in columns 1, 2, and 3 respectively; xylem shaded; compare table I.

and that the two gantry crane house plants were not so old in their last pictured stage as all other series.

In buckwheat there is better correspondence in the stages, since only two analyses were made, with diagrams at these stages.

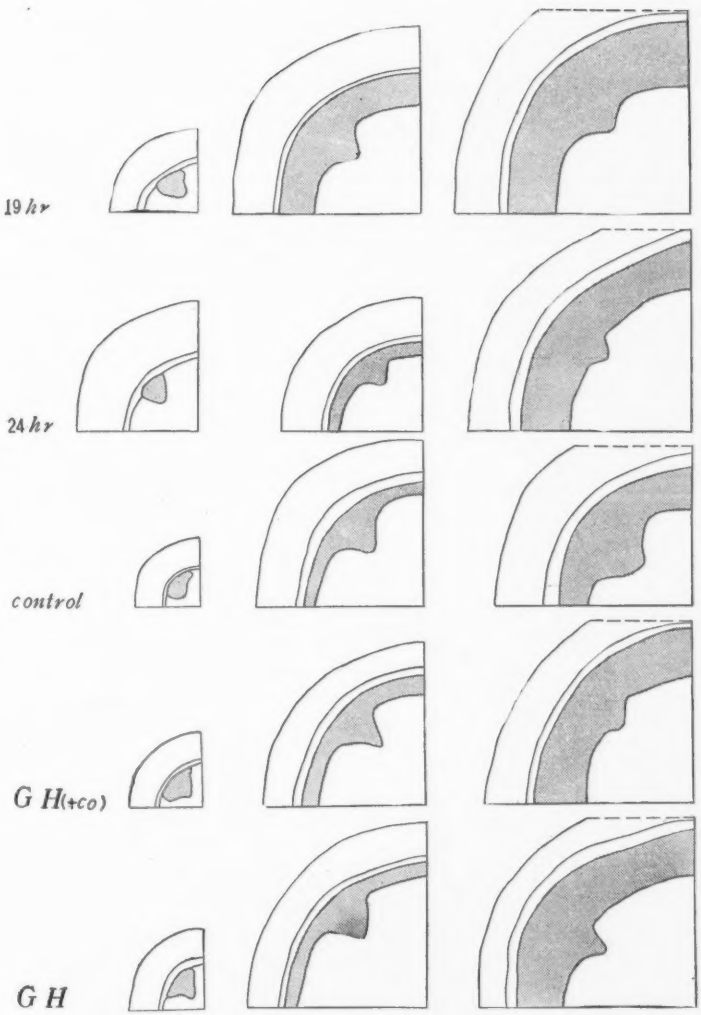


FIG. 2.—Diagrams of bases of stems of tomatoes grown in daily light durations of 19 and 24 hours, in control house (normal light of season), and in gantry crane houses with extra carbon dioxide and usual atmosphere; first, second, and third columns correspond, in 19 and 24 hour sets, to first, third, and fourth microchemical analyses respectively; in control, to first, second, and fourth microchemical analyses; in gantry crane houses, to first, second, and third microchemical analyses; compare table I.

In tomato all series with light duration of 12 hours or more attain as great a diameter or greater than the control, regardless of the

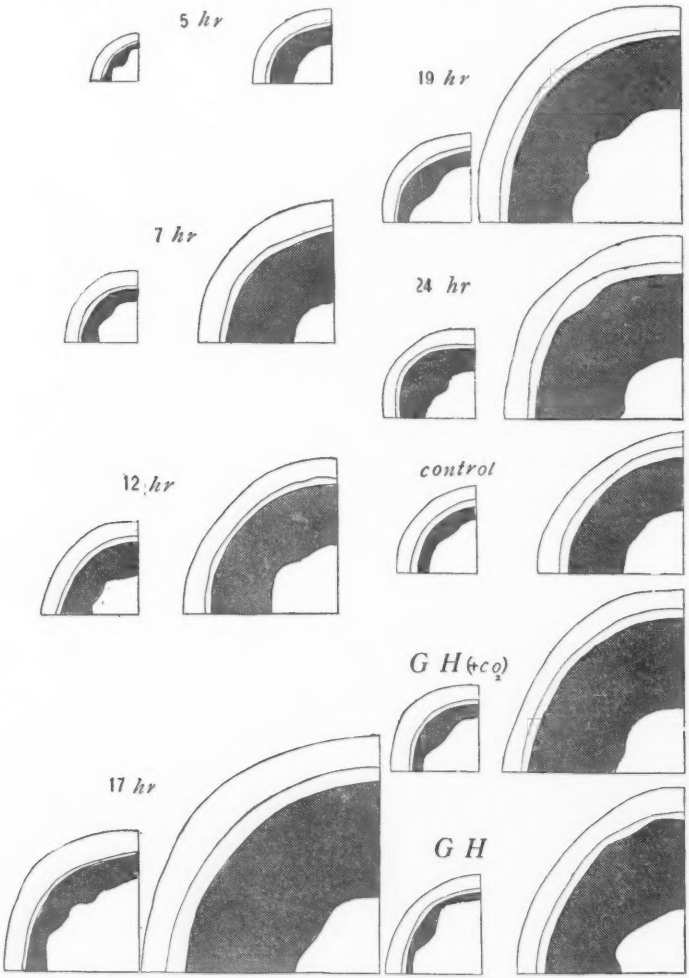


FIG. 3.—Diagrams of bases of stems of buckwheat in different sets of experiment: in each couple of diagrams the smaller shows condition at 17 days, larger at 48 days; compare table II with microchemical data.

success of the plant in other respects; but in several cases, as the 12 and 17-hour plants, there is a very pronounced development of xylem tissue, with relatively less cortex and pith contributing to the diameter. The 12-hour plant was rather diffuse in habit, with somewhat yellow-green leaves, and attained the greatest height of any series. Correlated with the chemical data, this series, having as reserves more carbohydrates and more protein than the lower series, was also utilizing more of these in upward growth than any other set, and gave good evidence in cross-sections of their utilization in highly differentiated rather than simple tissues.

The 17-hour plant, in comparison, although evidently producing much differentiated tissue, was only about three-fourths as tall, had lost all its lower leaves up to the seventh internode, and had a decidedly yellow aspect in the small bladed leaves remaining. It was evidently suffering, as were the plants with longer light durations, from the conditions under which it grew. At the time of cessation of the experiment it showed more carbohydrate reserve, but less protein than the 12-hour form. Considering the condition of the leaves, it is entirely likely that the plant would soon have reached the stage which the continuous light plant attained at about the termination of the run, and succumbed. The 19-hour and continuous light plants were successively shorter than the 17-hour, with progressively poorer physical appearance, as evinced by loss of leaves, yellowing, and, in the case of the 24-hour exposure, by the production of brown areas on leaves, petioles, and stems. Analyzed, these patches (fig. 20) show a sort of cork structure; sometimes similar brown patches are found inside the stem tissues. These give a cellulose reaction when tested, and fail to show tannins.

The gantry crane house plants showed somewhat similar results in tissue development when compared with one another, but the plants supplied with extra carbon dioxide were taller than the others. Compared microchemically, the latter had less reserve protein than the former, and both had only moderate reserve carbohydrates.

As might be expected, the plants with only 5 or 7 hours of light were smaller, very open in habit, with small blades as compared with the total size of the leaves. They were also very watery, and the thin leaves wilted readily. There was little development of the character-

istic complex oil that gives the plant its odor, in these or in the plants with 17, 19, or 24 hours of light.

In buckwheat the maximum diameter was attained in the plants grown in 17 hours of light. These obviously developed the greatest amount of xylem, and showed the greatest height, except for the plant in the gantry house with the usual atmosphere, which made it a close second. The shorter exposures produced correspondingly shorter plants, and correspondingly less of the highly differentiated tissues. Longer exposures show a similar succession from this maximum, although in less degree. The 19-hour and continuous light plants are somewhat shorter than the 17-hour, but almost twice as tall as the 7-hour plant. As compared with one another, there was a slight difference in the 19 and 24-hour plants in favor of the latter as regards both height and amount of xylem, which I believe to be due to variation in individuals rather than to direct effect of light.

The gantry crane house plants showed a slightly better development of xylem and bast when provided with extra carbon dioxide. On comparison with the rest of the series, they show less development than the 17-hour plant, and correspond fairly well with those receiving 19 or 24 hours of light.

In relating these facts with the microchemical data, the 5, 7, and 12-hour plants, like the control, showed low carbohydrate and low protein reserves, while those in longer exposures showed much carbohydrate and low protein. At the time of the last analysis all plants were in flower, but only those in 7 and 12 hours had set fruit. It is clear that the buckwheat attains maximum height and development of tissues and reserves in 17 hours' light exposure, in contrast with tomato, which, although attaining maximum tissue differentiation and reserves, fails to make the growth in height and total diameter. In summation of all points observed, the tomato is more successful in 12 hours' exposure.

LEAF STRUCTURE.—*Capsicum annuum* (variety Ruby King), *Coleus* sp., *Glycine Soja* var. Tokyo, Peking, Mandarin, and Biloxi, *Lactuca sativa* var. Mignonette, *Mirabilis Jalapa*, *Nicotiana Tabacum* var. Imported Havana, *Pelargonium* sp., *Solanum Melongena* var. Black Beauty, *Tropaeolum majus* (Grant Flowering Salmon Queen), *Lycopersicum esculentum* var. Bonny Best, and *Viola* sp. were here

considered. In addition, *Brassica oleracea* var. *capitata* (Early Jersey Wakefield) was used in determining the number of stomata.

Measurements of mature leaves in similar positions on the plants were made by means of an ocular micrometer on free-hand sections (table IV). In pepper, coleus, lettuce, tomato, four o'clock, geranium, and nasturtium, continuous light tended to reduce the thickness of the leaf blade to a greater or less degree, as compared with control plants (pl. XV). The effect of increase in length of light period to 19 hours in the gantry crane houses did not produce such uniform results as these. Pepper, coleus, lettuce, tobacco, and three varieties of soy bean (Tokyo, Peking, and Mandarin) showed a decrease in leaf thickness in both houses, whereas geranium, eggplant, nasturtium, tomato, violet, and Biloxi soy bean were thicker leaved in both houses. Four o'clock leaves were evidently thinner in the gantry crane house with ordinary atmosphere, but only very slightly thicker in that supplied with extra carbon dioxide.

When the comparison is made between plants in the two gantry crane houses, it is found that in two cases, nasturtium and Mandarin soy bean, the leaf thickness is the same under the two sets of conditions. In the majority of cases (nine), however, the reduction in leaf thickness is greater in the house where no carbon dioxide is supplied, while in three (tobacco, tomato, and lettuce) the reverse is true. This would tend to show that in the greater number of forms considered, the longer duration favored reduction in leaf thickness, which the increase in carbon dioxide content served to counterbalance in part. The number of forms and the number of individuals considered, however, do not warrant any sweeping generalizations.

In making measurements of leaves, attention was also given to the development and length of the palisade cells (table IV). As one might anticipate, usually there is a correlation, the palisade cells being shortest where the leaf is thinnest, and longest where it is thickest, so that there is shorter palisade in the thinner leaves in continuous light. To this generalization there are occasional exceptions, as the four o'clock and tomato in the gantry crane house with extra carbon dioxide. Obviously, where there is greatest decrease in thickness, the length of cells in the palisade layer may be so reduced that it is little differentiated in size from the adjacent

spongy cells (as coleus in continuous light); or, where a second palisade layer is usually present, this may be lacking (geranium in continuous light), or less marked (Peking soy bean in gantry houses).

TABLE IV

THICKNESS OF LEAVES AND LENGTH OF PALISADE CELLS

		CONTROL (MM.)	CONTINUOUS LIGHT (MM.)	GANTRY CRANE HOUSE, $+CO_2$ (MM.)	GANTRY CRANE HOUSE, USUAL ATMOSPHERE (MM.)
Capsicum annuum	Leaf	0.28	0.26	0.22	0.208
	Length	0.12	0.087	0.086	0.086
Coleus sp.	Leaf	0.27	0.14	0.21	0.18
	Palisade	0.06	0.046	0.07	0.049
Glycine Soja var. Biloxi	Leaf	0.35	.....	0.45	0.346
	Palisade	0.096	.....	0.157	0.14
		0.054	.....	0.06	0.06
var. Mandarin	Leaf	0.48	.....	0.40	0.40
	Palisade	0.20	.....	0.10	0.115
		0.10	.....	0.09	0.08
var. Peking	Leaf	0.43	.....	0.25	0.21
	Palisade	0.14	.....	0.07	0.05
		0.11	.....	0.04	0.04
var. Tokyo	Leaf	0.34	.....	0.32	0.26
	Palisade	0.085	.....	0.085	0.065
		0.09	.....	0.06	0.065
Lactuca sativa	Leaf	0.236	0.138	0.18	0.208
Lycopersicum esculentum	Leaf	0.27	0.24	0.35	0.48
	Palisade	0.12	0.08	0.10	0.10
Mirabilis Jalapa	Leaf	0.27	0.26	0.277	0.22
	Palisade	0.095	0.08	0.064	0.05
Nicotiana Tabacum	Leaf	0.28	.....	0.22	0.27
	Palisade	0.09	.....	0.08	0.065 double palisade
					0.055
Pelargonium	Leaf	0.229	0.225	0.33	0.31
	Palisade	0.05	0.059	0.08	0.08
		0.038	No 2nd palisade	0.06	0.04
Solanum Melongena	Leaf	0.22	.....	0.26	0.23
	Palisade	0.08	.....	0.16	0.11
Tropaeolum majus	Leaf	0.15	0.13	0.17	0.17
	Palisade	0.047	0.037	0.05	0.05
Viola sp.	Leaf	0.17	.....	0.21	0.18
	Palisade	0.07	.....	0.09	0.076

In regard to the epidermis, specific measurements were not made. From general observations, no marked trend was noted, nor were marked variations evident in the spongy tissue of leaves under different conditions. In the houses with additional light beyond solar light there was an evident increase in hairiness (soy beans), and a yellow appearance to the leaf rather than the good green of the check

plants. In this latter feature, the plants in the usual atmosphere are intermediate in coloring between the control and those with extra carbon dioxide. Evidently the prolonged light brings about some yellowing, which is aggravated by the increase in carbon dioxide. That there is some difference in size and distribution of chloroplasts is clear even to the casual observer, but the data are inadequate for a clear presentation of these points.

STOMATA.—Counts were made to determine the number of stomata on both leaf surfaces in six forms grown in four different situations. Table V shows the calculations for a square millimeter surface based on the averages from counts of thirty fields. The forms are too few to attach great weight to the results, but the latter may be utilized as a starting point for more thorough studies in later experiments. In continuous light, there are approximately uniform results, usually with reduction in number of stomata on both surfaces (table V). In the gantry crane houses there is sometimes an increase, sometimes a decrease, but usually the results are in the same direction for both houses and for both surfaces. This would seem to show that light is more effective as a factor in determining the number of stomata than is carbon dioxide. The gantry crane houses had similar light conditions, but different in intensity as well as duration, from those in continuous light. There is therefore no good basis of comparison for these two sets.

TABLE V

COUNTS OF STOMATA PER SQ. MM. OF LEAF SURFACE; AVERAGE OF THIRTY FIELDS

	Surface	Control	Continuous light	Gantry crane house, $+CO_2$	Gantry crane house, usual atmosphere
Brassica oleracea var. capitata	Upper	116	87	149	69
	Lower	161	155	207	126
Capsicum annuum	Upper	42	15	60	61
	Lower	113	97	232	187
Lactuca sativa	Upper	27	39	62	99
	Lower	41	77	47	120
Mirabilis Jalapa	Upper	62	12	83	65
	Lower	150	136	152	266
Pelargonium sp.	Upper	26	15	21	44
	Lower	148	67	68	121
Solanum Melongena	Upper	195	.....	85	130
	Lower	242	.....	204	221

An attempt was made to correlate the number of stomata with thickness of leaves. In the majority of cases where both were determined, there seemed a tendency to an increase in the number of stomata where the leaves were thinner. The number of cases, however, is too small for generalizations.

ROOTS.—Roots of tomato and buckwheat were examined at the time of study of other organs. Although there was not enough material for intensive work, and pot conditions are apt to cause greater variation in root systems than in aerial, nevertheless there was evident correlation with the development of the base of the stem. Since a plant with a fleshy root might give valuable additional data because of storage material, four o'clock roots were examined at the close of the experiment. The short period plants then showed low carbohydrate and low protein reserves and a small volume (as measured by displacement of water). There was a marked increase in volume in 17 and 19-hour plants, which in turn were smaller than the gantry crane house plant with usual atmosphere, the continuous light, and the other gantry crane house plant, which represented the maximum root development. The increase in diameters of roots was roughly comparable with volume, with 0.35 cm. at top for the 5-hour plant and 1.0 cm. for the 7-hour plant. In longer exposures, however, the simple root of low durations gave way to branched, very fleshy systems.

In contrast to the root systems, the aerial part attained the greatest height in 17 hours' exposure, next successively in (1) 19 hours, (2) gantry crane house with increased carbon dioxide, (3) other gantry crane house, and (4) continuous light and 12 hours, which were approximately equal. Text fig. 4 shows the volumes of roots and heights of stems, indicating that a conclusion in regard to the manufacture of materials, based on the stem height alone, would have been very misleading in this form.

DISCUSSION

A comparison of these results with those of previous workers brings out both similarities and contrasts. In leaves, continuous light produces thinner leaves with less pronounced palisade cells as seen by BONNIER (6). This fails to substantiate MASSART'S (20) conclu-

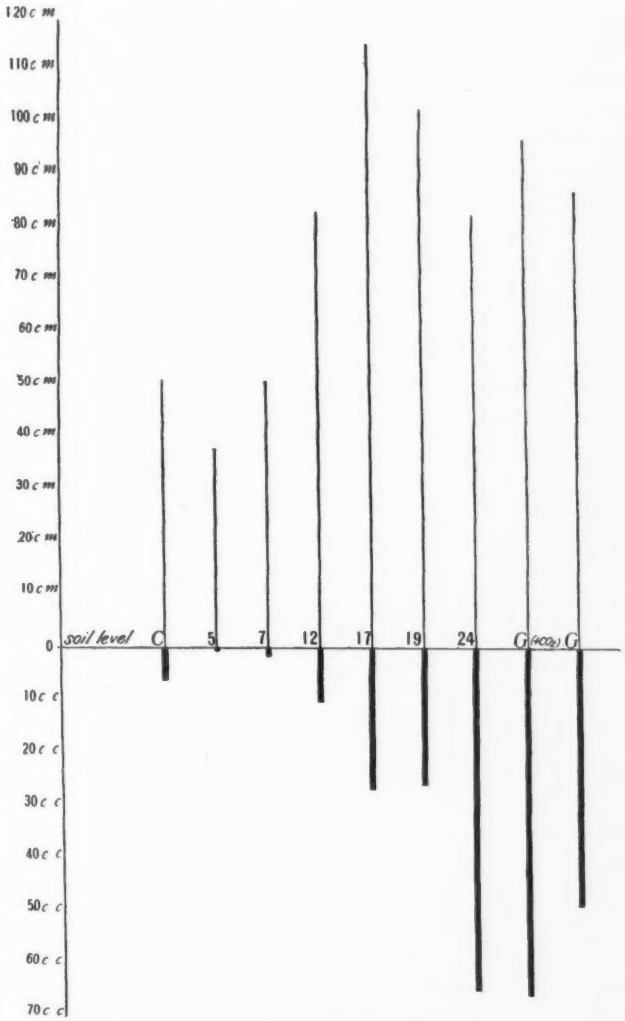


FIG. 4.—Relative development of stem and root regions of four o'clock; lines above "soil level" show height of stem in cm., lines below show volume of roots in cc., successively in control set, in daily light durations of 5, 7, 12, 17, 19, and 24 hours, and in gantry crane house with extra carbon dioxide and with normal atmosphere.

sions in regard to the general similar effect of 24, 18, 12, or indeed 6 hours' illumination on form and structure of leaves. MASSART believes that intensity is more effective in producing structural and form changes, and it may be that differences in intensity are responsible for differences in results obtained by investigators. The earlier records usually include the type of lamp used, range of distances of plants from lamp, but not the measurement of the light falling on the plant.

In long day plants in the gantry crane houses, thinner leaves were produced in the pepper, but thicker in the tomato. The latter is in accord with the work of Miss DEATS (**8**) on a similar form, the former is not. The presence of extra carbon dioxide might be a factor in one of these houses, but is not active in the other. Further work with more specimens may explain the difference in results.

In regard to stomata, no special work is on record of counts made under similar conditions. A comparison with the results of Miss ECKERSON (**11**) shows a difference in the control plants, due to conditions of growth in all likelihood. DUFOUR (**10**) has shown the result of greater intensity of light to be greater number of stomata. In my experiment, the continuous light plant had the lowest light intensity and the lowest number of stomata. Some gantry crane house plants, however, with equal intensity to the control plants during normal daylight plus an additional period of artificial light, also showed reduction in number, tending to remove responsibility from the intensity factor. The matter is complicated by the carbon dioxide supply, which makes an exact comparison with previous work impossible. In correlating with thickness of leaf, I find the usual result in most cases, the larger number of stomata in thinner leaves.

In the stem I failed to find the simplification of tissues reported by BONNIER. There was better agreement with the results of Miss DEATS, who found variation in amount of xylem in short day plants (6.5 hours), normal day plants, and long day (17.5 hours), in direct accord with the length of day. This carries no inference that further increase in time of exposure would further increase the amounts of xylem, under other similar conditions. Indeed my results show for the forms considered that there is a period of exposure for each form, beyond which additional duration is ineffective in producing differ-

entiated tissue, or increased height. The best interval for producing differentiation and height in tomato in artificial light of intensity used was 12 hours, in buckwheat 17 hours.

LUBIMENKO and SŽEGLOFF (18), in working with a range of shorter exposures (from 14 hours down to 4), found a greater depth of greenness in the longer durations, as did Miss DEATS in her 17.5-hour plants. General observations indicated lower chlorophyll content in the 5 and 7-hour plants than in the intermediate durations, with yellowing occurring to greater or less degree in the 19 and 24-hour exposures. Contrary to this last, BONNIER found greater amounts of chlorophyll in his continuous light plants. The majority of his plants were different from mine, it is not known that he used comparable intensity, and his experiments did not introduce the extra supply of carbon dioxide as a factor. These factors may account for differences.

Summary

1. In plants with short exposures to light, there are usually low carbohydrate and low protein reserves with less total growth and less production of differentiated tissues than in longer intervals.

2. In plants with longer light duration, there is increase in carbohydrate reserves without proportionately increased use in elaboration of proteins and tissue production.

3. Nitrates are present in great amounts in all tomato plants except the control and gantry crane house specimens. They are low in amount in buckwheat in 17, 19, and 24-hour exposures. They are usually lowest in the gantry crane house with extra carbon dioxide.

4. The maximum development of the plant (considering height and differentiated tissue) occurs in the 12-hour tomato and the 17-hour buckwheat.

5. Injury is evident in the tomato in exposures of 17 hours or more, resulting in marked decrease in photosynthetic ability.

6. Continuous light tends to produce thinner leaves, with palisade layer shorter or lacking.

7. The effect of conditions in the gantry crane houses as regards leaf thickness is variable.

8. Thinner leaves usually show an increase in the number of stomata, except in continuous light in intensity used.

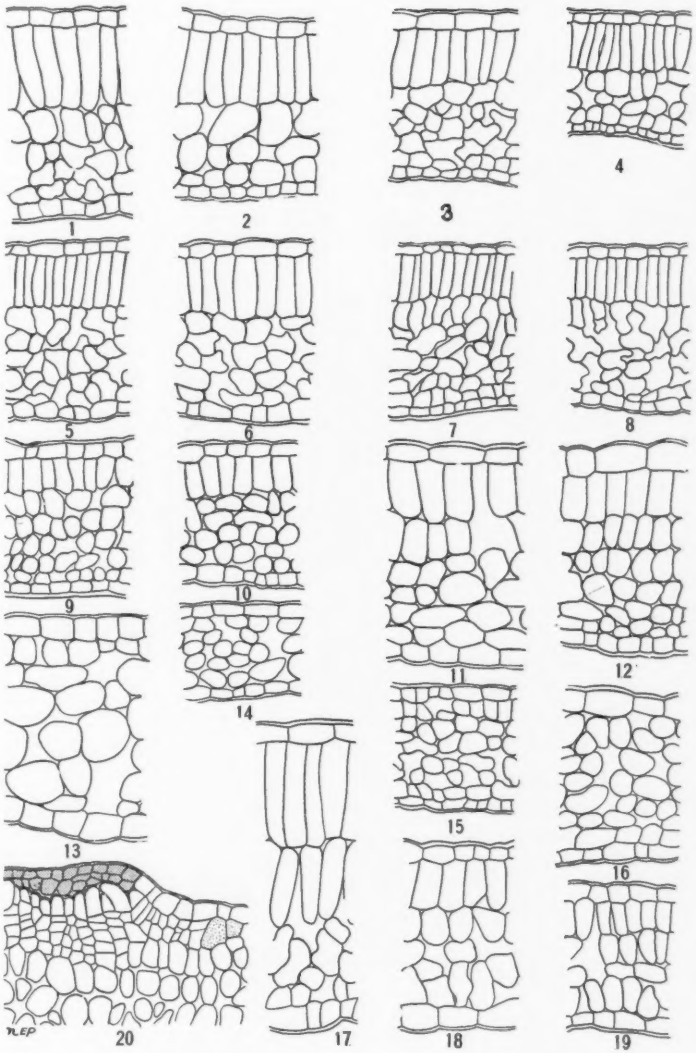
9. Root development in fibrous systems appears roughly comparable with that of the aerial parts.

10. In the storage roots of four o'clock, maximum development occurs in the gantry crane houses and continuous light, while maximum height of stem occurs in the 17-hour plant.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH
YONKERS, N.Y.

LITERATURE CITED

1. ADAMS, J., Relation of flax to varying amounts of light. BOT. GAZ. **70**:153–156. 1920.
2. ———, Effect on certain plants of altering daily period of light. Ann. Botany **37**:75–94. 1923.
3. ———, Some further experiments on the relation of light to growth. Amer. Jour. Bot. **12**:398–412. 1925.
4. ARTHUR, J. M., The effect of controlled environmental conditions on plants (unpublished).
5. BAILEY, L. H., Some preliminary studies of the influence of the electric arc lamp on greenhouse plants. Cornell Agric. Exp. Sta. Bull. 30:85–122. 1891.
6. BONNIER, G., Influence de la lumière électrique continue sur la forme et la structure des plantes. Rev. Gén. Bot. **7**:241–257; 289–306; 332–342; 409–419. 1895.
7. CORBETT, L. C., A study of the effect of incandescent gas light on plant growth. W.Va. Agric. Exp. Sta. Bull. 62. 79–110. 1899.
8. DEATS, M. E., The effect on plants of the increase and decrease of the period of illumination over that of the normal day period. Amer. Jour. Bot. **12**: 384–392. 1925.
9. DEHÉRAIN, P. P., Ann. Agronom. **7**:551. 1881; **14**:281. 1888. Rev. by BAILEY, Cornell Agric. Exp. Sta. Bull. 30. 119, 120. 1891.
10. DUFOUR, L., Influence de la lumière sur la forme et la structure des feuilles. Ann. Sci. Nat. Bot. V. **7**:311–413. 1887.
11. ECKERSON, SOPHIA H., The number and size of stomata. BOT. GAZ. **46**:221–224. 1908.
12. GARNER, W. W., and ALLARD, H. A., Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agric. Res. **18**:553–606. 1920.
13. ———, Further studies in photoperiodism. Jour. Agric. Res. **23**:871–920. 1923.
14. GARNER, W. W., BACON, C. W., and ALLARD, H. A., Photoperiodism in relation to hydrogen-ion concentration of the cell sap and the carbohydrate content of the plant. Jour. Agric. Res. **27**:119–156. 1924.
15. HARVEY, R. B., Growth of plants in artificial light. BOT. GAZ. **74**:447–451. 1922.



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16. HAYDEN, J. L. R., and STEINMETZ, C. P., Effect of artificial light on growth and ripening of plants. Gen. Electr. Rev. **21**:232–236. 1918.
17. HENDRICKS, E., and HARVEY, R. B., Growth of plants in artificial light. II. Intensity of continuous light required for blooming. BOT. GAZ. **77**:330–334. 1924.
18. LUBIMENKO, M. V., and SŽEGLOFF, O., Sur l'adaptation des plantes à la durée de la période claire de la journée. Compt. Rend. Acad. Sci. Paris **176**:1915–18. 1923.
19. MANGON, H., Production de la matière verte des feuilles sous l'influence de la lumière électrique. Compt. Rend. Acad. Sci. Paris **53**:243–244. 1861.
20. MASSART, J., L'action de la lumière continue sur la structure des feuilles. Acad. Roy. Belg., Bull. Sci. Ser. V. **6**:37–43. 1920.
21. NIGHTINGALE, G. T., Light in relation to the growth and chemical composition of some horticultural plants. Proc. Amer. Soc. Hort. Sci. **19**:18–29. 1922.
22. OAKLEY, R. H., and WESTOVER, L. H., Effect of the length of day on seedlings of alfalfa varieties and the possibility of using this as a practical means of identification. Jour. Agric. Res. **21**:599–607. 1921.
23. PRILLEUX, E., De l'influence de la lumière artificielle sur la réduction de l'acide carbonique par les plantes. Compt. Rend. Acad. Sci. Paris **69**:408–412. 1869.
24. SIEMENS, C. W., On the influence of electric light upon vegetation, and on certain physical principles involved. Proc. Roy. Soc. London **30**:210–219; 293–295. 1880.
25. TIEDJENS, V. A., Stimulation of plant growth by means of electric lighting. Ann. Convent. Ill. Eng. Soc. October 1924.
26. WANN, F. B., Some of the factors involved in the sexual reproduction of *Marchantia polymorpha*. Amer. Jour. Bot. **12**:307–318. 1925.
27. WANSER, H. M., Photoperiodism of wheat; a determining factor in acclimatization. Science N.S. **56**:313–315. 1922.

EXPLANATION OF PLATE XV

FIGS. 1–4.—Pepper leaf: successive figures show structure in control, continuous light, and gantry crane house with extra carbon dioxide, and with usual atmosphere.

FIGS. 5–8.—Four o'clock leaf: successive figures as in pepper.

FIGS. 9–12.—Geranium leaf: successive figures as in pepper.

FIGS. 13–16.—Lettuce leaf: successive figures as in pepper.

FIGS. 17–19.—Soy bean var. Peking leaf: successive figures show control, gantry crane house with extra carbon dioxide, and with usual atmosphere.

FIG. 20.—Outer portion of tomato stem, showing injury in continuous light: shaded portion brown in living material; stippled cells are subepidermal ones which have retained green coloring; corklike cells between brown layer and collenchyma tissue.

GROWTH OF SEEDLING IN RELATION TO COMPOSITION OF SEED

MARY E. REID

(WITH PLATES XVI–XVIII)

Experiments with seedlings varying in their proportions of carbohydrates to nitrogen have been conducted by allowing the seedlings to grow on their own nitrogen reserves to maximum size in light, in darkness, and in light in atmospheres containing 0.4 per cent CO_2 and lacking CO_2,[1] except for small amounts given off in respiration. Corresponding tests have also been conducted in which the seedlings have been given nitrate nitrogen. The seedlings were grown in sterilized quartz sand, kept moist by the use of nutrient solutions. The object was to study the total growth-producing value of the foods stored in the different seeds, and the relations of varying proportions of these organic foods in altering the shoot to root ratios. Of particular interest have been the relations of total nitrogen to growth, and the modifying influences of additional carbohydrates, synthesized in the light, and of nitrate nitrogen on seedlings grown in light and in darkness. All records of growth are based on measurements of size and green weights. In calculating the efficiency of the food reserves for growth, the seed coats were removed.

1. Seedlings grown in darkness at 21°–24° C.

(*a*) ON THEIR OWN NITROGEN RESERVES.—The food reserves having a high proportion of nitrogen and fat have been the most efficient in producing growth in darkness. The total growth in grams of green material produced per gram of original dry food material ranged from 4.86 gm. for low-protein corn to 32.51 gm. for sunflower, a high-protein, high-oil seed. The shoot to root ratios ranged from 2.01 for low-protein wheat to 8.37 for sunflower. Similar differences between varieties occur as between species; for example, high-protein corn and high-protein wheat both have higher

[1] The measurements of CO_2 concentration were made by Dr. WARD B. DAVIS.

shoot to root ratios than the corresponding types that are low in protein.

(*b*) WITH NITRATES.—Only a few of the seedlings, and these to only a small extent, responded to nitrates with an increase in total growth. Nitrates increased the growth of high-protein seedlings in as many instances as in seedlings of low-protein seeds. In nearly all cases there has been some modification of the proportions of shoots to roots, the seedlings receiving nitrates having relatively the greater weight of shoots. It seems that, although nitrates in most cases do not increase the quantity of growth in darkness, they do have the capacity for modifying the type of response.

2. Seedlings grown in atmospheres containing different amounts of CO_2

The atmospheres used contained high concentrations (0.4 per cent) of CO_2 and no CO_2, except for small amounts given off in respiration, the experiment being conducted during the latter part of May and early in June. The average temperature was 25° C. Seedlings of each type were harvested when the culture of the particular type, which had the most limiting conditions for growth, had reached its maximum size. Cultures of each kind, grown with and without CO_2, were harvested on the same day.

(*a*) ON THEIR OWN NITROGEN RESERVES.—Seedlings from low-protein corn, when grown in an atmosphere lacking CO_2 except for small traces given off by the seedlings themselves in respiration, produced larger plants with greener leaves than did those which had 0.4 per cent CO_2. Seedlings from high-protein corn, however, grew more and had greener leaves in an atmosphere containing 0.4 per cent CO_2. Seedlings from other types of high-protein seeds, such as soy bean, cowpea, sunflower, and cantaloupe, all responded similarly to the high-protein corn, and were also greener when given CO_2 than when grown without it. Some kinds of seedlings had their total growth increased nearly 150 per cent by using CO_2. Even more striking than the effect of CO_2 on total growth, is its influence on the shoot and root ratios. Growth of roots was increased much more than growth of shoots in those seedlings whose total growth was increased by CO_2. Growth of roots of soy bean seedlings was increased

202 per cent more than that of the tops. From these results it seems that CO_2 must have had a modifying influence in the utilization of foods stored in the seed.

(*b*) WITH NITRATES.—Seedlings from low-protein seeds, such as low-protein corn, when grown on their own carbon supply, had their growth increased by the addition of nitrates. Seedlings from high-protein seeds, grown on their own carbon supply, varied in their behavior with respect to the addition of nitrates. Sunflower seedlings grew the same amount with nitrates as without, and muskmelon, cowpea, and soy bean seedlings grew somewhat more with nitrates. When grown in high concentrations (0.4 per cent) of CO_2, seedlings of both high and low-protein seeds showed increased growth with nitrates, but those of high-protein seeds had the greater increase. The response in total growth of seedlings of high-protein seeds to CO_2 in most cases was much greater than their response to nitrates. The response in total growth of seedlings of low-protein seeds to CO_2 was much less than their response to nitrates.

3. Seedlings grown in light in normal atmosphere

This experiment was conducted in October, the plants being grown in the greenhouse in daylight at an average temperature of 24° C. There were many cloudy days during the progress of the experiment. Each kind of seedling was allowed to grow until the seedlings of the lot grown without nitrates had reached their maximum size. The seedlings grown with and without nitrates for each type used were harvested at the same time.

(*a*) ON THEIR OWN NITROGEN RESERVES.—In most cases there was a greater total growth in light than there had been in darkness in the experiments previously described. Low-protein wheat, however, grew as much in darkness as in light; low-protein corn grew 45 per cent less in light than in darkness; and rice grew 43 per cent less in light than in darkness. Seedlings from high-protein seeds, on the contrary, grew much more in light than in darkness, and some of them produced blossom buds. Greater than the differences in total growth, however, were the differences in shoot to root ratios, relatively more roots being produced in light than in darkness. The greatest degree of shifting of ratios occurred in the seedlings of high-

protein seeds. With the exception of seedlings of the Gramineae, there were notable increases in leaf development in the light, as indicated by size, weight, and number of leaves.

(*b*) WITH NITRATES.—The seedlings may be classified into three groups with respect to their response to nitrates: seedlings of legumes, seedlings from low-protein and high-protein starchy seeds, and seedlings from high-protein, oily seeds. Seedlings of the leguminous type were the least responsive to nitrates. In general, they did not produce more than 30 per cent more total growth with nitrates than without, under the conditions of light and temperature used in this experiment. In previous experiments, conducted during the longer and sunnier days of May and early June, it had been shown that leguminous seedlings lacking nodules did have the ability to utilize nitrates. The tests with soy bean and cowpea seedlings, however, had indicated that increase of growth with nitrates did not occur to any extent except when photosynthesis of carbohydrates could go on at the same time. For this reason it is supposed that the failure to respond to nitrates during the shorter and somewhat cloudy days of October may be a seasonal condition, and may be connected with the lower light intensity and its resulting effects on photosynthesis of carbohydrates and proteins. Whatever may be the cause, whether difference in light intensity and duration, or temperature, or a combination of these factors, the fact remains that the legumes behaved in this experiment as a group that is characterized by a low grade ability to utilize nitrates.

Seedlings of the low-protein starchy seeds, all members of the grass family, in general produced the greatest response to nitrates in a given time. Low-protein corn gained 194 per cent more than high-protein corn as a result of using nitrates. Low-protein wheat gained 85 per cent more with nitrates than did high-protein wheat. Rice was injured by nitrates. The seedlings grew much larger without nitrates than with them. Seedlings of the high-protein, high-oil type responded more slowly to the influence of nitrates. During the first 10 days of growth, the seedlings with and without nitrates were not greatly different in size. Tomato and sunflower seedlings were exceptions; they showed well marked differences in the earlier stages of growth. It is supposed that the behavior of sunflower

seedlings is explainable because of their rapid response to CO_2 as discovered in a preceding experiment. It was then noted that sunflower seedlings responded remarkably to CO_2 even when nitrates were lacking, but that there was no response to nitrates when CO_2 was lacking. Tomato seedlings have not yet been tested in this way. At the time the plants were harvested, the seedlings of the high-protein, high-oil type presented notable differences when grown with and without nitrates. This was to some extent because they were allowed to grow for a longer time than seedlings of the low-protein, starchy type. In a future experiment it is planned to obtain the weights of the entire lot of seedlings at 5, 10, 15, and 20 day intervals. In this way it is hoped to present the quantitative and qualitative differences in the growth responses at different stages in the development of the seedlings of the various types studied.

With the exception of cotton seedlings, the shoot to root ratios of all types were increased by the addition of nitrates. In general, those seedlings whose total growth was most markedly influenced by nitrates were the ones whose shoot to root ratios showed the greatest increases. Seedlings of the Leguminosae were the least responsive in this respect, as they also were in their response in total growth.

The growth responses of the seedlings to varying proportions of carbohydrates to nitrogen agree with results obtained with tomato cuttings, described in former papers.[2]

Summary

1. Different kinds of seedlings produce very different growth responses when exposed to the same external influences. The responses are often related to the chemical composition of the seed.

2. If the nitrogen content of the seed is low in proportion to the carbohydrates, the response as measured by an increase in total growth in terms of green weight is greater when nitrates but no CO_2 (except traces given off in respiration) are utilized than when CO_2 but no nitrates are utilized.

[2] REID, MARY E., Relation of the kind of food reserves to regeneration in tomato plants. BOT. GAZ. 77: 103–110. 1924.

———, Quantitative relations of carbohydrates to nitrogen in determining growth responses in tomato cuttings. BOT. GAZ. 77: 404–418. 1924.

———, Growth of tomato cuttings in relation to stored carbohydrate and nitrogenous compounds. Amer. Jour. Bot. 13: 1926 (detailed report).

3. If the nitrogen content of the seed is high in proportion to the carbohydrates, the response as measured by an increase in total growth is greater when CO_2 but no nitrates are utilized than when nitrates but no CO_2 (except for traces given off in respiration) are utilized.

4. The greatest total amount of growth, however, is produced by seedlings from both high and low-nitrogen seeds by allowing the seedlings to utilize both nitrates and CO_2.

5. The utilization of both nitrates and CO_2 tends to result in a shifting of the relative amounts of shoots and roots, that is, there are qualitative as well as quantitative responses to these external influences. The proportion of shoots to roots of low-protein seeds tends to be increased because of the relatively larger increase in the amount of shoots, if nitrates but no CO_2 is utilized; whereas the proportions of shoots to roots of seedlings of high-protein seeds are not increased and in some cases are even decreased by this treatment.

6. The shoot to root ratios of seedlings of low-protein seeds are not noticeably affected if CO_2 but no nitrates are utilized, but the shoot to root ratios of high-protein seeds tend to be greatly reduced if CO_2 but no nitrates are utilized. This shifting of ratios is a result of a greater increase in the amount of roots than of shoots.

7. The proportions of shoots to roots of seedlings of low-protein seeds tend to be slightly increased if both CO_2 and NO_3 are utilized, but the proportions of shoots to roots of seedlings of high-protein seeds are very greatly decreased by such treatment, because the weight and number of roots increase much more than the shoots.

8. The modification of type of growth is correlated with extent of modification in quantity of growth. Those treatments which result in the greatest increase in quantity of growth are the ones which produce the greatest change in type of growth.

(The foregoing statements apply to growth of seedlings in the light.)

9. A high-protein, high-oil food supply appears to be the most efficient in producing growth both in darkness and in light. This is not surprising, since both types of foods are constituted of a relatively large proportion of the condensed compounds of their respective types. For example, high-protein seeds contain relatively much

more of basic nitrogen and consequently have a great amount of nitrogen in proportion to their weight; oils contain a great amount of carbon in proportion to their weight.

10. The shoot to root ratios of seedlings grown in darkness appear to vary with the proportion of nitrogen to carbohydrates in the food reserves of the seed, the higher the nitrogen in proportion to carbohydrate content the higher the shoot to root ratios of the seedlings. There is some evidence that addition of nitrates to the culture medium of the seedlings grown in darkness can modify the type of growth (increase shoot to root ratios), but in only a few cases do nitrates increase the amount of growth.

11. Since wide variations in growth responses are associated with variations in the types and quantities of storage materials in the seed, it seems inadvisable to draw conclusions as to the effect of mineral nutrients or carbon dioxide treatment upon the subsequent growth of the plants when the observations are based on growth responses during the first three weeks of growth only. Before studying the effect of an element or compound on growth (in general), it would seem desirable to conduct experiments in which the storage supply of the element or compound in question has been at least partially exhausted, by allowing the seedlings to grow to maximum size without that substance. This method has been employed by animal workers for some time.

12. Although contradictory results have not been found in the different series of experiments, the results are not considered conclusive but will be repeated and elaborated.

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YONKERS, N.Y.

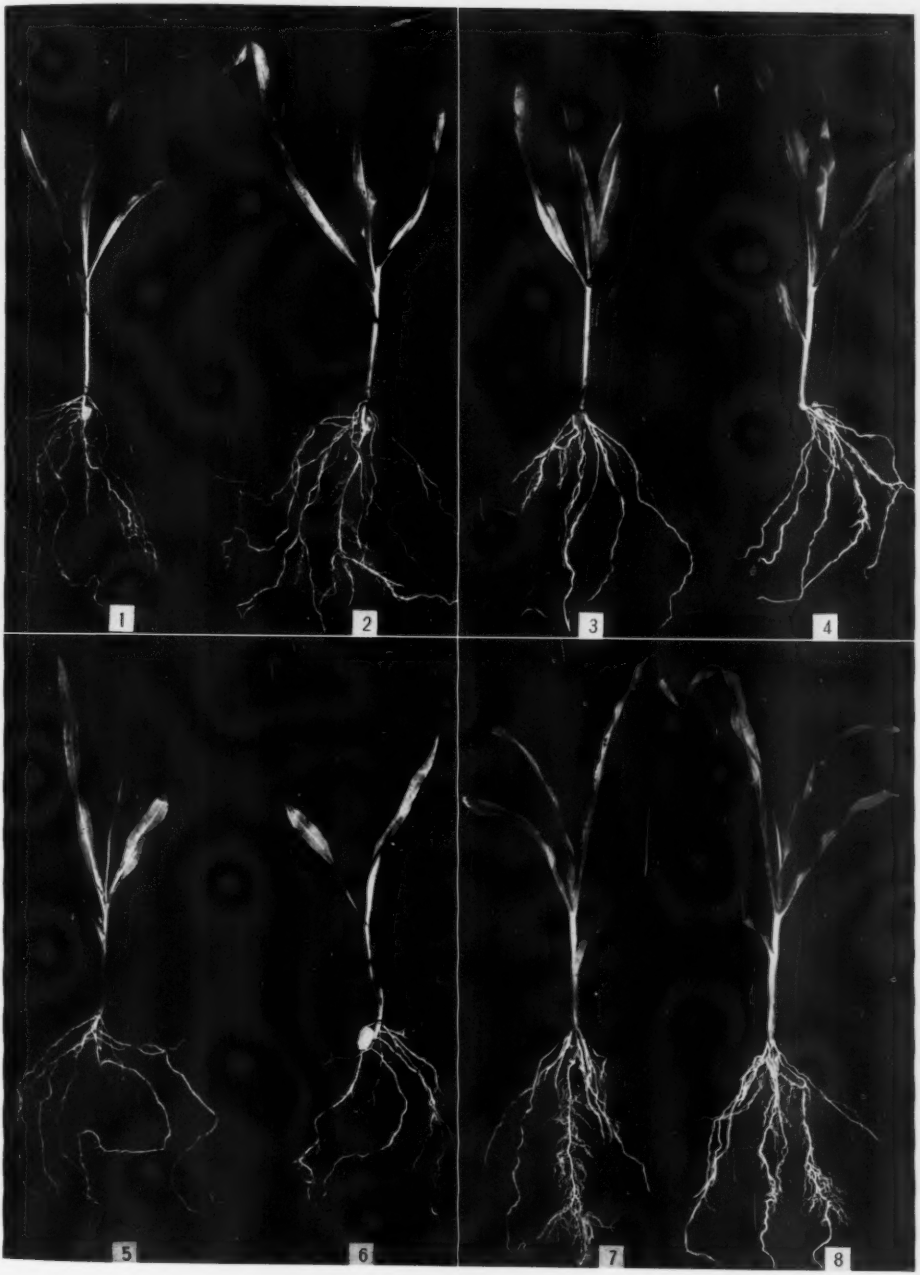
EXPLANATION OF PLATES XVI–XVIII

PLATE XVI

FIGS. 1, 2.—Illinois high-protein corn seedlings, grown without nitrates: former without CO_2, latter with CO_2.

FIGS. 3, 4.—Illinois high-protein corn seedlings grown with nitrates: former without CO_2, latter with CO_2.

FIGS. 5, 6.—Illinois low-protein corn seedlings grown without nitrates: former without CO_2, latter with CO_2.



REID on SEEDLING GROWTH

REID on SEEDLING GROWTH

REID on SEEDLING GROWTH

FIGS. 7, 8.—Illinois low-protein corn seedlings grown with nitrates: former without CO_2, latter with CO_2.

PLATE XVII

FIGS. 9, 10.—Rocky Ford melon seedlings grown without nitrates: former without CO_2, latter with CO_2.

FIGS. 11, 12.—Rocky Ford melon seedlings grown with nitrates: former without CO_2, latter with CO_2.

FIGS. 13, 14.—New Era cowpea seedlings grown without nitrates: former without CO_2, latter with CO_2.

FIGS. 15, 16.—New Era cowpea seedlings grown with nitrates: former without CO_2, latter with CO_2.

PLATE XVIII

FIGS. 17, 18.—Low-protein (Little Club) wheat seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

FIGS. 19, 20.—High-protein (Marquis) wheat seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

FIGS. 21, 22.—New Era cowpea seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

FIGS. 23, 24.—Hubbard squash seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

AN ORGANISM OF TOMATO MOSAIC

SOPHIA H. ECKERSON

(WITH PLATES XIX–XXII)

Introduction

This study was begun in 1922 at the University of Wisconsin, in connection with work on wheat rosette with H. H. MCKINNEY, while on part-time appointment from the Cereals Division of the United States Department of Agriculture. The early studies of tomato mosaic were made in 1922 and 1923, with the cooperation of E. J. KRAUS of the University of Wisconsin. Dr. KRAUS also turned over to me abundant material, both diseased and healthy plants, for the study of mosaic in *Hippeastrum Johnsoni* and pepper. The figures in pl. XIX were drawn from slides made at that time. The work has been continued at Boyce Thompson Institute. At present H. R. KRAYBILL and the writer are starting a study combining differential filtration, inoculation, and microchemical examination, which should bring out new facts about mosaic. In the meantime, I shall describe organisms seen in mosaic plants, especially the one in tomato, which has been observed most carefully.

Motile organisms

WHEAT.—Motile organisms were first seen in the rosette wheat. In the first few days of the infection there were numerous tiny flagellated forms (2–4 μ) in the cells. A week later there were fewer of these, but many larger motile forms (5–7 μ). Still later the large non-motile bodies predominated. These different forms always appeared in this sequence.

TOMATO.—An examination of mottled leaves of mosaic plants available revealed motile organisms in all. The very young, not yet mottled leaves of the mosaic plants also had hundreds of tiny rapidly moving organisms (fig. 5 right) in the mesophyll cells; while in the phloem cells were elongated forms having typical flagellate movement. In the older, badly mottled leaves were many melon-seed-

shaped, sporelike forms having a hyaline membrane (fig. 8 right). These were imbedded in the few remaining chloroplasts as well as free in the cells. These sporelike forms were puzzling, since they were not stainable, either living or in smears. More is known about them now, but they are still somewhat puzzling.

VITAL STAINS.—Much time was given to observation of the living organisms within the living cells of the plant. Of the many vital stains tested for differentiation, the combination of methylene blue and eosin was the most satisfactory for the small forms. These small ovoid forms, having amoeboid movement (fig. 6), and the long thin flagellate forms stain readily. When the stains are used in very dilute solution, the organisms are tinged with the eosin, while their nuclei are blue. Recently I have used polychrome methylene blue instead of the eosin combination. Since the nuclei of these small organisms are denser than the somewhat transparent body, they can be seen clearly in living unstained condition by proper arrangement of the light.

Brilliant cresyl blue is satisfactory for the larger forms (fig. 7 left). If used in combination with acid fuchsin, the organisms are tinged with red, while their nuclei are blue. This combination was also used with good results on wheat rosette for distinguishing the big bodies from the cell nuclei. In that case the stains were made up in formalin.[1] The bodies were pink with blue nuclei. The cell nuclei were blue with pink nucleoles. This was excellent for diagnosis, but the colors were not permanent.

Sections of fresh tissue were stained also with iron haematoxylin and with azur-eosin under observation at the microscope. This was for localization of the organisms, and to be certain that the ones stained in the smears were those observed living in the cells.

ORGANISMS IN CHLOROPLASTS.—This year Dr. KRAYBILL and I have inoculated several series of young tomato plants with filtered juice from mosaic plants. Alternate leaflets of three or four leaves on each plant were scratched with a fine needle, and the juice brushed over them. I examined the uninoculated leaflets, opposite

[1] (1) 0.25 gm. brilliant cresyl blue, 90 cc. 4 per cent formalin, and 10 cc. methyl alcohol; (2) 0.05 gm. acid fuchsin, 0.05 gm. orange G, 95 cc. 4 per cent formalin, and 5 cc. 1 per cent acetic acid. Mix a few drops of (1) and (2); stain lightly (about 10 min.).—Enzlyk Mik. Tech. 1910.

those inoculated, at intervals of twenty-four hours for the first few days.

Twenty-four hours after inoculation, in leaflets opposite inoculated leaflets, there were tiny flagellated organisms (fig. 13 and fig. 15 extreme left) in the veins and in the adjacent mesophyll cells. None were found in other regions. Most of the chloroplasts of these cells were in healthy condition. Many had several small starch grains at the surface (fig. 9 left), but in a few of the cells nearest the veins the chloroplasts were beginning to show signs of dissolution. Small organisms appeared to be entering some of these plastids (fig. 9 middle), while they had entered and were beginning to swim around in little pools in others (fig. 9 right).

Three days after inoculation there were tiny flagellated forms, and also somewhat larger forms (fig. 14) in great numbers throughout the mesophyll tissue.

Five days after inoculation many cells of the mesophyll tissue were in bad condition. Their chloroplasts were no longer held by the cytoplasm in orderly arrangement, but were floating free. Most of these chloroplasts showed progressive liquefaction, while the organisms within them were larger (fig. 10 left and middle). Many of the chloroplasts were almost wholly liquefied (fig. 10 right and fig. 11 left).

Seven days after inoculation some chloroplasts of the palisade cells were in process of liquefaction. Many of the remaining plastids contained non-motile bodies which seemed to be early stages of spore formation (fig. 11 middle and right).

Ten days after inoculation some of the leaflets were beginning to show mottling. Within the leaf groups of palisade cells were partially disorganized; the cytoplasm was gone (or liquefied); and the chloroplasts were in disarray, many partially dissolved, others containing spores (fig. 12). These groups of disorganized cells were usually bounded by groups of cells apparently in perfectly healthy condition.

The foregoing description is from our latest series. The specific number of days after inoculation for any stage varied a little in the different series, but the sequence was the same in all. Moreover, the sequence in inoculated plants is the same as that from the youngest

leaf of any mosaic plant down to the fifth or sixth from the tip. The only difference found was in the later stages of the disease. In plants growing under good cultural conditions in the field, there are fewer spores but many more big slowly motile forms (fig. 16).

SPORES.—Twenty to thirty days after inoculation, although there were still groups of good cells, most of them had become filled with melon-seed-shaped spores. These cells were completely disorganized, without either cytoplasm or chloroplasts. They were like little boxes filled with pebbles which spilled out when the walls were cut.

The mature spores (fig. 8 right) have a highly refractive, hyaline membrane of slight permeability, and are difficult to stain. Recently, however, rather good results have been obtained by fixing in methyl alcohol, staining overnight in iron haematoxylin, and destaining.

Before the spores are mature, that is before the hyaline membrane is formed, the walls are very permeable. Their contents are difficult to stain in place, because they are shot out during fixation. Many spore cases show a spiral filament still attached. There are always dozens of detached tails on the slide, and among these many tiny flagellated forms. On one particularly good slide the tiny organisms were in such a position on the filament that it seemed that they must have been shot out together (fig. 18).

Peculiar bodies, appearing much like Japanese lanterns in the living tissue, were caught in two of the inoculated series (fig. 17 left two figs.; fig. 18 left). When stained with haematoxylin they show clearly two or three organisms within each. These tiny organisms are the same type as those present at the beginning of the disease (fig. 13 left).

Organisms in other mosaic plants

For comparison with tomato mosaic, several other plants were examined. I found similar organisms in all, although not necessarily the same organism. There are slight differences in form and considerable differences in size, but the general appearance and behavior are the same. There are tiny organisms in the youngest leaves, also in inoculated plants of *Hippeastrum Johnsoni* (fig. 2). There are elongated flagellate forms in the phloem tissue of older leaves (*Hip-*

peastrum Johnsoni, fig. 3; *Dahlia*, fig. 22 left; squash, fig. 25 left). There are also the larger slow moving forms (*H. Johnsoni*, fig. 4 left; squash, fig. 26 left). In badly mottled leaves of all there are spore forms (*Dahlia*, fig. 23 right; squash, fig. 26 right; *H. Johnsoni*, fig. 4 right).

The spores from *Dahlia* should be good material for a study of development, structure, contents, and germination of these, as yet little understood bodies. I hope to make such a study next summer.

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EXPLANATION OF PLATES XIX-XXII

All figures were drawn with a Bausch & Lomb 1.8 mm. fluorite objective, 1.3 N. A., and Leitz periplane ocular 20X. All magnifications are approximately 3200 diameters.

PLATE XIX

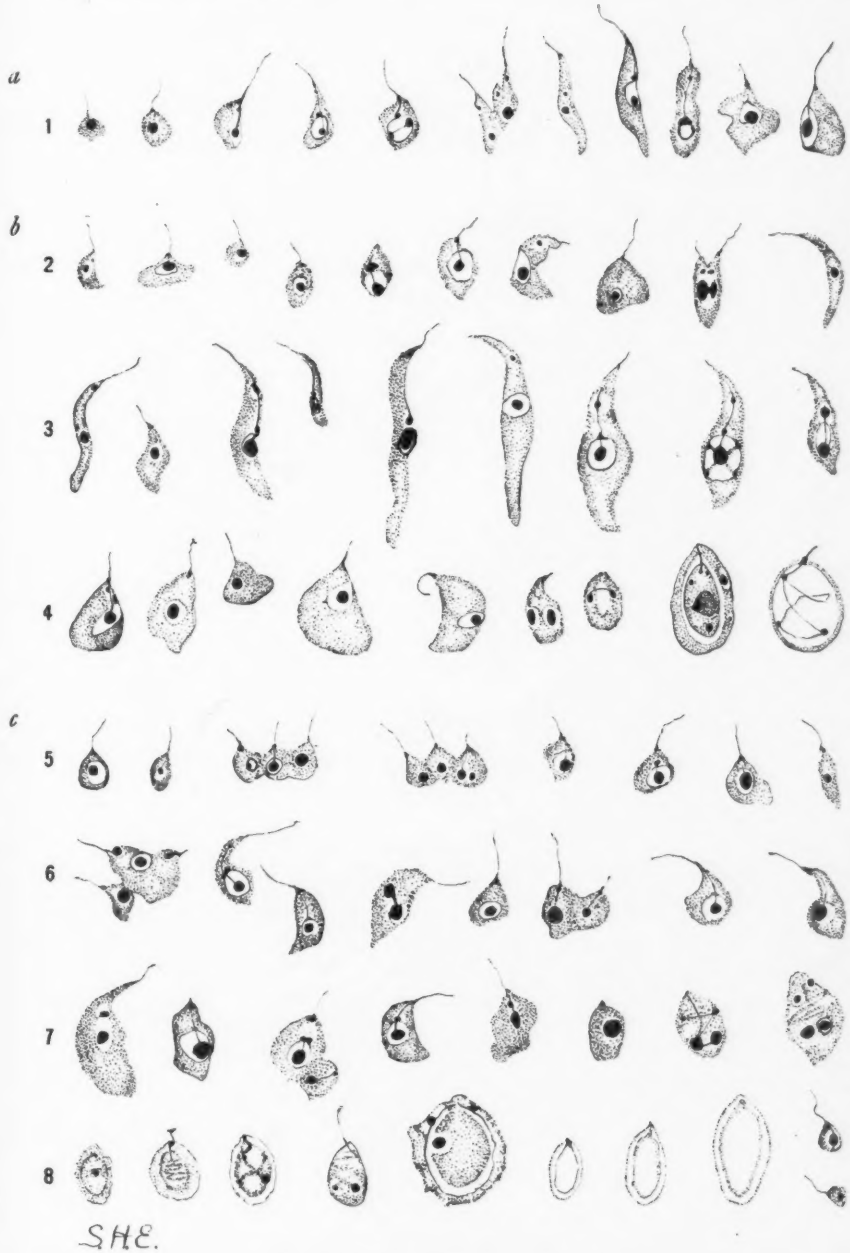
FIG. 1.—Pepper, with iron-alum haematoxylin: tiny flagellated forms; elongated forms; at right, amoeboid forms.

FIGS. 2–4.—*Hippeastrum Johnsoni*, with iron-alum haematoxylin: fig. 2, from young leaf of seedling 10 days after inoculation; fig. 3, elongated flagellate forms from phloem of leaf of mature plant; fig. 4, from badly mottled leaf; at right, stages in spore formation.

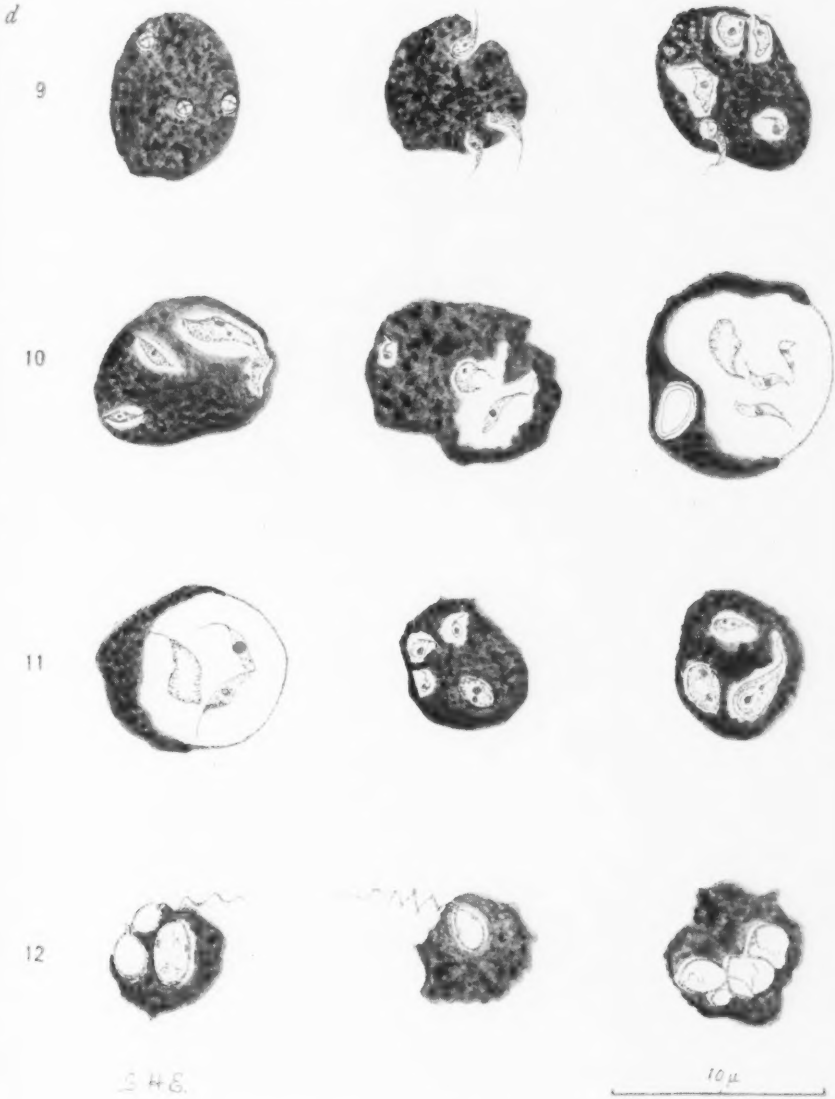
FIGS. 5–8.—Tomato, with azur-eosin: fig. 5, from youngest leaf of mosaic plant, division; fig. 6, from young mottled leaf; fig. 7, three figs. at right probably stages in spore formation; fig. 8, stages in spore formation; at right three spores with hyaline membranes; a few tiny flagellated forms found on slide among these spores.

PLATE XX

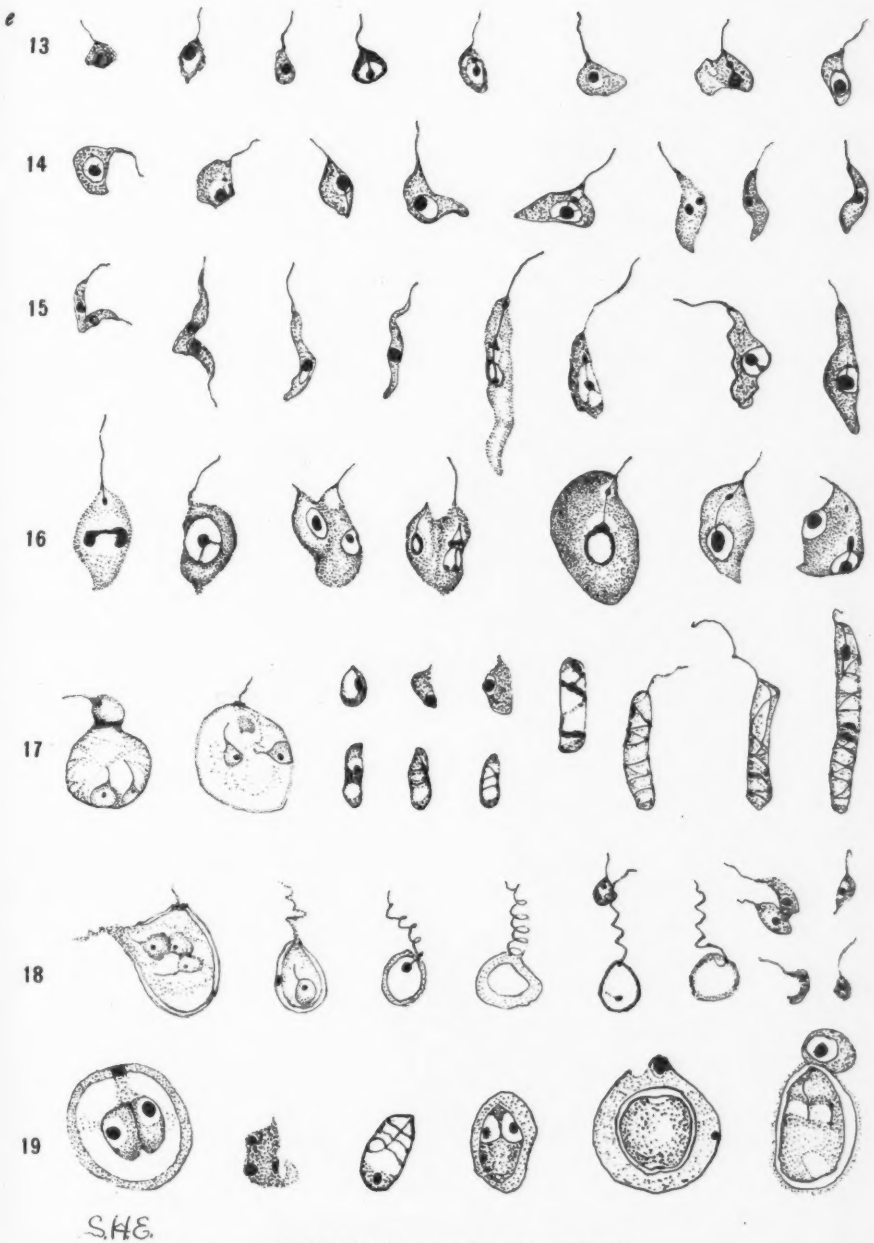
FIGS. 9–12.—Tomato, chloroplasts from leaflets opposite inoculated leaflets; with two exceptions fixed in 1 per cent osmic, stained with iron haematoxylin 10 minutes at 45° C.: fig. 9, from mesophyll cells bordering veins, 24 hours after inoculation; left figure with small starch grains, which show clear cross in polarized light; middle figure, tiny organisms entering plastid; right figure, organisms in plastid, which is beginning to liquefy; fig. 10, from mesophyll cells 5 days after inoculation; increasing liquefaction of plastid; right figure from living unstained tissue; nuclei seen by oblique light; nucleus not visible in the denser organism; spore with hyaline membrane imbedded in remainder of plastid; fig. 11, left figure similar to right figure of fig. 10; middle and right figures from palisade cells 7 days after inoculation; fig. 12, from palisade cells 10 days after inoculation; leaflet slightly mottled; spiral filaments shot out by osmic acid.



ECKERSON on TOMATO MOSAIC



ECKERSON on TOMATO MOSAIC



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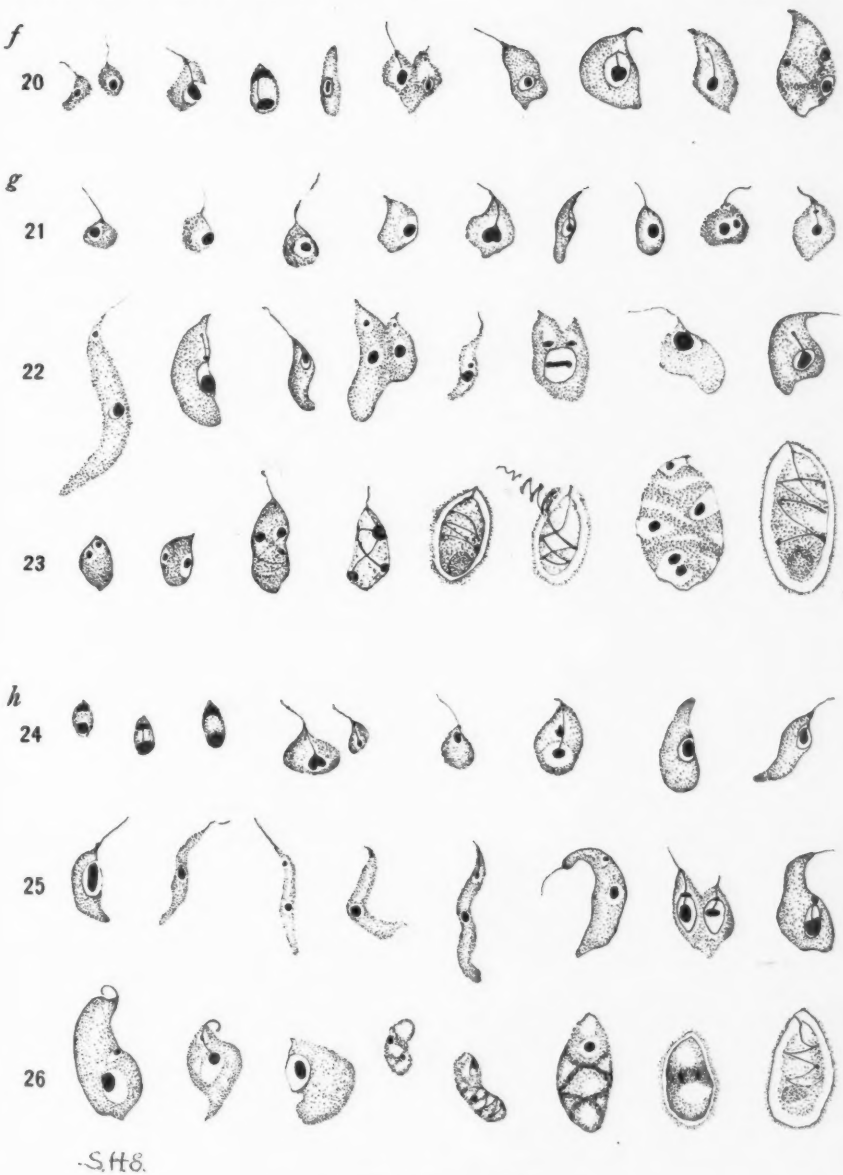

ECKERSON on TOMATO MOSAIC

PLATE XXI

FIGS. 13–19.—Tomato: fig. 13, tiny flagellated forms having rapid buzzing movement, predominating in youngest leaf, also in leaf the first few days after inoculation; fig. 14, larger forms having active amoeboid movement, found in second and third leaf below tip, also in leaf third and fourth days after inoculation; fig. 15, elongated forms having typical flagellate movement, found chiefly in phloem parenchyma; fig. 16, larger less active (except when dividing) forms from fifth and sixth (from tip) mottled leaves from large plants in garden; fig. 17, two figures at left from palisade cells 5 days after inoculation; figures at right probably spore forms, from phloem cells; fig. 18, two figures at left containing tiny organisms similar to those in fig. 13; spores having spiral filaments, extruded during fixation; at extreme right tiny flagellate forms seemingly ready to start the cycle anew; fig. 19, stages in formation of large spores, found chiefly in badly mottled leaves from garden.

PLATE XXII

FIG. 20.—Strawflower: figures at leaf from youngest leaf; at right from mottled leaf; figure at extreme right beginning spore formation (?).

FIGS. 21–23.—Dahlia: fig. 21, from yellow tip of stunted badly diseased plant; fig. 22, from older mottled leaf; fig. 23, from large mottled leaf of vigorous shoot, stages in spore formation.

FIGS. 24–26.—Squash: fig. 24, from young leaf of badly diseased and distorted plant; fig. 25, elongated flagellate forms; fig. 26, from badly mottled leaf; at left large forms having slow movement; at right spore formation.

ATTEMPT TO CULTIVATE AN ORGANISM FROM TOMATO MOSAIC[1]

HELEN A. PURDY

Introduction

In discussing the etiology of diseases of man and animal produced by filterable viruses, the consensus of opinion is in favor of the theory that they are caused either by microorganisms not demonstrable by our present methods, or by ultramicroscopic organisms (**9, 10, 13, 18**). This conclusion is based upon the fact that the active agent in the filtrate apparently multiplies within the living host, since an infection obtained by inoculation with the filtrate can be transferred successively through a series of animals after the dilution of the original inoculum has exceeded the point capable of reproducing the disease. Although the virus of tobacco mosaic exhibits the same property of indefinite multiplication within living plants, not all plant pathologists regard this evidence as sufficient support for the parasitic theory of mosaic diseases.

Many hypotheses have been proposed to explain the origin of tobacco mosaic as a purely physiological response to unfavorable environmental conditions of the soil or climate (**3, 5, 7, 14, 15**), or due to various agents such as a "contagium vivum fluidum" (**1**), unorganized ferments or toxins (**6, 7**), oxidizing enzymes (**2, 4, 16, 17**), and only recently to potato protoplasm (**8**). It seems likely that all of these hypotheses would be superseded by the parasitic theory, if the virus could be multiplied *in vitro*.

Many unsuccessful attempts have been made to cultivate a causative organism from plants affected with mosaic disease. Considerable interest, therefore, was aroused when OLITSKY (**12**) recently reported success in cultivating *in vitro* an active agent that would produce mosaic disease in tobacco and tomato plants. The importance of OLITSKY'S conclusions makes a careful repetition of his experiments desirable. The investigations here reported were

[1] The writer is indebted to Dr. L. O. KUNKEL for valuable suggestions and a critical review of the manuscript.

undertaken with the purpose of repeating his recent work. With a few modifications, the methods employed by OLITSKY were carefully duplicated. While this work was in progress, MULVANIA (11) reported that he had repeated OLITSKY'S work, but was unable to obtain any evidence that the active agent of mosaic disease multiplies outside the living tobacco and tomato plants.

The stock virus used was extracted from mosaic-affected tomato plants in a greenhouse of the Boyce Thompson Institute. Although OLITSKY obtained his tomato mosaic virus from the same source a year previously, the writer has no proof that the two stock viruses were identical.

Method

PREPARATION OF MEDIUM.—Eighty gm. of tomato shoots, 5–6 weeks old, were minced with scissors and ground to a soft pulp in a sterile mortar; 250 cc. of distilled water added, and the entire mixture centrifuged at high speed for one hour. The supernatant fluid was passed, first through a paper pulp suction filter to remove the bulk of plant tissue, then through a sterile Berkefeld "W" under strictly aseptic conditions, and finally through a second similar Berkefeld filter.[2] The hydrogen-ion concentration of the filtrate was determined by the electrometric method. The reaction of the various lots of medium gave a range of P_H 5.08–6.26.[3] The medium was tubed and incubated at 28°–30° C. for 7 days. At the end of this period of incubation, all tubes showing evidence of contamination or a precipitation of albumins and globulins were discarded. Only the tubes that contained clear media were used.

TESTING OF MEDIUM.—At the end of a 7-day period of incubation, a tube of medium was inoculated into 16 healthy tomato plants. After a second week of incubation, the inoculation of 16 additional healthy plants was repeated. If the 32 plants inoculated with a given lot of medium remained healthy, the medium was considered free from virus.[4]

CULTURE.—The stem of a badly affected mosaic tomato plant

[2] OLITSKY used a Berkefeld "N" and no paper pulp suction filter.

[3] The media used by OLITSKY gave a reaction of P_H 5.3–6.0.

[4] Considerable difficulty was experienced in obtaining medium free from virus.

was cut with a razor. The cut end was flamed, and a sterile capillary pipette was inserted in the stem, from which approximately 0.01 cc. of juice was withdrawn and inoculated into 5 cc. of medium. The culture was then incubated at 28°–30° C. After 7–14 days, subplants were made by adding 0.5 cc. of the original culture, thoroughly rolled to insure mixing, to 5 cc. of medium. Subsequent subplants were made in a similar manner, and the approximate dilution of the introduced virus was estimated.

INOCULATION.—Vigorous tomato plants about 6 weeks old were inoculated by scarifying one leaf on each of three separate branches of the same plant and rubbing in the inoculum with a cork. Cognizant of the fact that a plant, apparently healthy, might already be affected with mosaic disease in its incipient stages, every precaution was taken throughout inoculation to safeguard against a chance transmission of mosaic from one plant to another. The leaves to be inoculated were held by means of a separate paper slip for each plant; the needle used for scarifying the leaves was sterilized by flaming after each inoculation; and an individual sterile cork was employed for rubbing in the inoculum. Moreover, all inoculated plants, including those used for control, were held together for four weeks in the same greenhouse, which was kept carefully fumigated.[5] Under these conditions, all of the plants were exposed equally to the risk of accidental infection.

CONTROLS.—In every experiment, in addition to the medium inoculated with virus, two tubes, each containing 5 cc. of medium from the same lot, were included. One was uninoculated; the other inoculated with 0.01 cc. of juice from a healthy tomato plant. These control tubes were treated in every way like the virus cultures. Subplants were made, using the same lot of medium to which the corresponding virus culture had been transplanted. A tube containing 5 cc. of sterile tap water inoculated with 0.01 cc. of juice from a mosaic affected tomato plant was also included in every experiment.[6] Subplants of the water culture corresponding to those of the virus cultures were made in sterile tap water.

[5] Thanks are due FREDERICK E. HEINSOHN for cooperation in keeping the greenhouse free from insect pests.

[6] MULVANIA introduced this control in his repetition of OLITSKY's experiments.

Results

Of the plants that were inoculated from virus cultures containing original inoculum in an estimated dilution of approximately 2×10^{-3}, 41 of 50, or 82 per cent developed mosaic disease (table I). Subplants from these cultures produced infection in 13 of 70, or 19 per cent of the plants receiving virus in a dilution amounting to 2×10^{-4}. Upon reaching the dilution 2×10^{-5}, the virus cultures infected 9 per cent, or 6 of the 70 plants inoculated. At the next subplant, a 2×10^{-6} dilution was obtained that proved incapable of producing mosaic in any of the 70 plants into which it was introduced.

Of the five separate virus cultures made, three lost their power of infectivity upon reaching a dilution of the original 0.01 cc. of virus approximating 2×10^{-5} or 1–50,000, while the remaining two produced no mosaic at the next subplant, which was approximately a 2×10^{-6} or a 1–500,000 dilution.

The two water cultures lost their power of infectivity at a dilution of the original 0.01 cc. of virus approximating 2×10^{-5} or 1–50,000[7] (table II).

A total of 284 plants used in the control experiments remained healthy. Some were inoculated with plain medium; others with medium inoculated with juice from a healthy tomato plant.

Discussion

In the tabulated results it will be noted that the power of infectivity of each original virus or water culture and of every subplant in each series was tested and recorded. By this method, any appreciable multiplication of the active agent of mosaic disease may readily be detected. Also, the power of infectivity of the virus cultures can unquestionably be attributed to the 0.01 cc. of virus introduced in the original cultures, since the results of the control experiments prove that all the culture media used were free from any trace of virus. Also, by testing the entire series of subplants of a given culture successively, accidental infection from other sources than contaminated medium can more easily be detected, since a culture producing infection at the fourth subplant, when the third was non-

[7] MULVANIA reported a higher percentage infection from his water cultures than from his virus cultures at a given dilution.

TABLE I

POWER OF INFECTIVITY OF VIRUS CULTURES AT VARIOUS DILUTIONS

CULTURE	PERIOD OF INCUBATION (DAYS)	P_H OF MEDIUM	APPROXIMATE DILUTION OF ORIGINAL VIRUS	NO. OF PLANTS INOCULATED	NO. OF PLANTS DISEASED*	NO. OF PLANTS HEALTHY*	PER CENT INFECTION	AVERAGE PER CENT INFECTION
A...	14	6.19	2×10^{-3}	10	10	0	100	
B...	14	6.19	2×10^{-3}	10†	2	2	50	
C...	7	5.69	2×10^{-3}	2	2	0	100	
D...	7	5.69	2×10^{-3}	2	1	1	50	82
E...	7	5.69	2×10^{-3}	2	2	0	100	
C...	14	5.69	2×10^{-3}	10	10	0	100	
D...	14	5.69	2×10^{-3}	10	6	4	60	
E...	14	5.69	2×10^{-3}	10	8	2	80	
A^1‡.	7	6.19	2×10^{-4}	10	5	5	50	
B^1..	7	6.19	2×10^{-4}	10	0	10	0	
A^1..	14	6.19	2×10^{-4}	10	4	6	40	
B^1..	14	6.19	2×10^{-4}	10	0	10	0	19
C^1..	14	6.19	2×10^{-4}	10	0	10	0	
D^1..	14	6.19	2×10^{-4}	10	2	8	20	
E^1..	14	6.19	2×10^{-4}	10	2	8	20	
A^2§.	14	5.69	2×10^{-5}	10	0	10	0	
B^2..	14	5.69	2×10^{-5}	10	0	10	0	
$1A^2$..	14	5.69	2×10^{-5}	10	4	6	40	
$1B^2$..	14	5.69	2×10^{-5}	10	0	10	0	9
C^2..	14	6.19	2×10^{-5}	10	2‖	8	20	
D^2..	14	6.19	2×10^{-5}	10	0	10	0	
E^2..	14	6.19	2×10^{-5}	10	0	10	0	
A^3..	14	5.69	2×10^{-6}	10	0	10	0	
B^3..	14	5.69	2×10^{-6}	10	0	10	0	
$1A^3$..	14	5.08	2×10^{-6}	10	0	10	0	
$1B^3$..	14	5.08	2×10^{-6}	10	0	10	0	0
C^3..	14	6.10	2×10^{-6}	10	0	10	0	
D^3..	14	6.10	2×10^{-6}	10	0	10	0	
E^3..	14	6.10	2×10^{-6}	10	0	10	0	
$1A^4$..	14	6.26	2×10^{-7}	10	2¶	8	20	
$1B^4$..	14	6.26	2×10^{-7}	10	0	10	0	
C^4..	14	5.90	2×10^{-7}	10	0	10	0	4
D^4..	14	5.90	2×10^{-7}	10	0	10	0	
E^4..	14	5.90	2×10^{-7}	10	0	10	0	
$1A^5$..	14	5.90	2×10^{-8}	10	0	10	0	
$1B^5$..	14	5.90	2×10^{-8}	10	0	10	0	
C^5..	14	5.90	2×10^{-8}	10	0	10	0	0
D^5..	14	5.90	2×10^{-8}	10	0	10	0	
E^5..	14	5.90	2×10^{-8}	10	0	10	0	
			Total	366				

* Final number recorded four weeks after inoculation.

† Six inoculated plants were discarded accidentally a few days after inoculation.

‡ A^1 is the first subplant of A, A^2 is a subplant of A^1, etc.

§ A^2 and $1A^2$ are both subplants of A^1. A^2 was made when A^1 had been incubated one week, $1A^2$ after A^1 had been incubated two weeks.

‖ Accidental?

¶ These plants were standing beside plants affected with mosaic; doubtless accidental infection since the culture was non-infectious at the preceding dilution.

infectious, would be a questionable result, requiring substantiation before the infection could definitely be attributed to virus present in the culture (table I, footnotes ||, ¶; table II, footnote §).

The fact that the two water cultures did not exhibit the high

TABLE II

POWER OF INFECTIVITY OF WATER CULTURES AT VARIOUS DILUTIONS

CULTURE	PERIOD OF INCUBATION (DAYS)	APPROXIMATE DILUTION OF ORIGINAL VIRUS	NO. OF PLANTS INOCULATED	NO. OF PLANTS DISEASED*	NO. OF PLANTS HEALTHY*	PER CENT INFECTION	AVERAGE PER CENT INFECTION
X	14	2×10^{-3}	10	2	8	20	9
Y	7	2×10^{-3}	2	0	2	0	
Y†	14	2×10^{-3}	10	0	10	0	
X^1‡	7	2×10^{-4}	10	0	10	0	6
X^1†	14	2×10^{-4}	10	0	8	0	
Y^1	14	2×10^{-4}	10	2§	10	20	
X^2||	14	2×10^{-5}	10	0	10	0	0
$1X^2$¶	14	2×10^{-5}	10	0	10	0	
Y^2	14	2×10^{-5}	10	0	10	0	
X^3	14	2×10^{-6}	10	0	10	0	0
$1X^3$	14	2×10^{-6}	10	0	10	0	
Y^3	14	2×10^{-6}	10	0	10	0	
$1X^4$	14	2×10^{-7}	10	0	10	0	0
Y^4	14	2×10^{-7}	10	0	10	0	
$1X^5$	14	2×10^{-8}	10	0	10	0	0
Y^5	14	2×10^{-8}	10	0	10	0	
		Total	152				

* Final number recorded four weeks after inoculation.

† This culture is a portion of the preceding one which has been incubated one week longer.

‡ X^1 is the first subplant of X, X^2 is a subplant of X^1, etc.

§ Accidental?

|| Subplant of X^1 after incubating X^1 for one week.

¶ Subplant of X^1 after incubating X^1 for two weeks.

power of infectivity that the virus cultures of corresponding dilutions showed, cannot be explained adequately by assuming on the one hand that the tap water had an inhibiting or perhaps deleterious effect upon the active agent of the fresh virus, or by claiming on the other hand a slight multiplication of the virus in the medium used in the virus cultures. The number of water and virus cultures tested is too small, and the method of introducing the original inoculum does not permit of enough accuracy for fair comparisons. More significant

conclusions can be drawn by comparing the percentage infection exhibited by successive subplants of the same series.

Conclusion

In the experiments here reported, in which the methods of OLITSKY were followed, the writer has been unable to obtain any evidence that the active agent producing mosaic disease in tobacco and tomato plants multiplies outside the living plants.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH
YONKERS, N.Y.

LITERATURE CITED

1. BEIJERINCK, M. W., Über ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. Verhandel. Kon. Akad. Wetensch. Amsterdam II. 6^5:1–22. *pls. 1, 2.* 1898. (Abstract in Centralb. Bakt. Zweite Abt. **5**:27–33. 1899).
2. CHAPMAN, G. H., Mosaic and allied diseases, with special reference to tobacco and tomatoes. Ann. Rep. Mass. Agric. Exp. Sta. **25**:94–104. 1913.
3. EGIZ, S. A., Tabakovodstvo. Glavnoe Upravlenïe Zemledîêliîâ i Zemleustroĭstva, Department Zemledîêliîâ, Obshchedostynnyîâ Soobshchenïîâ sel 'skokhozîâĭstvennykh Uchrezhdenïĭ i Spetsĭalistov po Sel 'skokhozîâĭstvennoi Chasti (Russia). no. 9. 1912.
4. HEINTZEL, KURT, Contagiöse Pflanzenkrankheiten ohne Microben, mit besonderer Berücksichtigung der Mosaikkrankheit der Tabaksblätter. Erlangen pp. 46. *pl. 1.* (Inaugural-Dissertation). 1900.
5. HUNGER, F. W. T., De mozaiek-ziekte bij Deli-tabak. Deel I. Verslag van de op Deli met betrekking tot de mozaiek-ziekte genomen proeven in de jaren 1901–1902. Mededeel. 'Slands Plantentuin **63**:1–104. 1902.
6. ———, Untersuchungen und Betrachtungen über die Mosaikkrankheit der Tabakspflanze. Zeits. Pflanzenkrank. **15**:257–311. 1905.
7. ———, Neue Theorie zur Ätiologie der Mosaikkrankheit des Tabaks. Ber. Deutsch. Bot. Gesells. **23**:415–418. 1905.
8. JOHNSON, JAMES, Transmission of viruses from apparently healthy potatoes. Wis. Agric. Exp. Sta. Res. Bull. 63. 12. 1925.
9. JORDAN, E. O., A textbook of general bacteriology. p. 589. 1924.
10. MCFARLAND, JOSEPH, A textbook upon the pathogenic bacteria and protozoa. p. 27. 1919.
11. MULVANIA, MAURICE, Cultivation of the virus of tobacco mosaic by the method of OLITSKY. Science N.S. **62**:37. 1925.

12. OLITSKY, P. K., Experiments on the cultivation of the active agent of mosaic disease in tobacco and tomato plants. Jour. Exp. Med. **41**:129–136. 1925.
13. PARK, W. H., and WILLIAMS, A. W., Pathogenic microorganisms. p. 530. 1920.
14. STURGIS, W. C., Preliminary notes on two diseases of tobacco. Conn. Agric. Exp. Sta. Ann. Rep. **22**:242–260. 1899.
15. ———, On the effects, on tobacco, of shading and the application of lime. Conn. Agric. Exp. Sta. Ann. Rep. **23**:252–261. 1899.
16. WOODS, A. F., The destruction of chlorophyll by oxidizing enzymes. Centralb. Bakt. Zweite Abt. **5**:745–754. 1899.
17. ———, Observations on the mosaic disease of tobacco. U.S. Dept. Agric., Bur. Pl. Ind. Bull. 18. 1–24. *pls. 1–6.* 1902.
18. ZINNSER, HANS, and RUSSELL, F. F., A textbook of bacteriology. p. 889. 1924.

TWO NEW SPECIES OF ZAMIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 345

CHARLES J. CHAMBERLAIN

(WITH SIX FIGURES)

Genera in the cycads are not only so distinct that they are easily recognizable, but they are so sharply defined that interrelationships within the family are uncertain. Sister MARY ALICE[1] constructed a key to the genera, based upon the leaflets. A similar key, impractical to apply, but nevertheless effective wherever material is available, could be based upon pollen tube structures. The usual keys, based upon cones, are very effective when cones are available.

Some species are almost as sharply limited as the genera; but others, especially in the larger genera, present such variation that identification is difficult, and it is likely that new species have been described when no new description was needed. In *Macrozamia* there is a plexus of forms, with *M. spiralis* as a center, which might afford a better study in variation than in taxonomy, for the description of species might degenerate into a description of individuals. There are similar centers in all the larger genera, especially in *Zamia*, which contains more than a third of all the species in the family. Consequently, one should exercise some caution in describing new species in cycads. Nevertheless, there are doubtless many new species of this family still waiting to be discovered and described. Two species of *Zamia*, from Mexico, are sufficiently distinct to be described as new. One I raised from a seed secured near Jalapa, and the other I dug up near Tuxtepec. The former has been under observation in my collection for nearly twenty years, and the latter for fifteen years.

Before venturing to describe these plants as new species, I examined all the *Zamia* material at Kew, both in the herbarium and in the greenhouses. I also had the pleasure of studying the collection in the greenhouse and herbarium of the New York Botanical Garden, doubtless the most extensive collection of *Zamia* in the world. Dr.

[1] LAMB, SISTER MARY ALICE, Leaflets of Cycadaceae. BOT. GAZ. 76:185–202. 1923.

N. L. BRITTON, Director of the Garden, went through the entire collection with me, giving the benefit of his extensive acquaintance with this genus in the field, and also of his long experience in taxonomy. Not being a taxonomist, I might have hesitated to describe the species without this assistance; but a description seemed necessary, since the species from the Jalapa neighborhood has been used in hybridizing, and is also being used in a cytological study of the determination of sex.

Zamia monticola

This species was grown from a seed collected on the steep mountain side about six or eight miles west of Jalapa, opposite the extinct crater of Naolinco. The mountain side is densely covered with shrubs and small trees, with a rich herbaceous undergrowth. A *Begonia* was found here with a leaf which measured a meter across. This is the best region for *Ceratozamia* encountered during several collecting trips in the Mexican tropics. *Ceratozamia* is very abundant on the steep slopes and it cones freely. In 1906, I picked up a great number of seeds of *C. mexicana*. Dr. C. R. BARNES and Dr. W. J. G. LAND were with me on that trip, and none of us noticed any *Zamia* in this region. When the seeds were planted, all the pots were marked *C. mexicana*, and not until the seedlings were well developed was *Ceratozamia* no. 10 noticed to be a typical *Zamia*.

On August 8, 1915, nearly eleven years from the planting of the seed, a male cone appeared, followed at intervals of about a week by three others. These were typical *Zamia* cones, appearing in succession, so that when the first cone was 8 cm. in length, exclusive of the peduncle, the others measured respectively 6, 4, and 3 cm. At the shedding stage, the first cone had reached a length of 16 cm., and its peduncle measured 17 cm. The other three cones were slightly smaller when they reached the shedding stage.

Since 1915 this plant has coned five times, producing four cones in 1918, six in 1920, six in 1922, two in 1923, five in 1924, and four in 1925. The cones are all large for the genus, the first cone in each set reaching a length of 12–16 cm. at the shedding stage. The peduncles, which are as long as the cones, are seldom erect; more often they are nearly horizontal and curved (fig. 1). Of the six cones produced in 1920, the first began to shed its pollen on December 14,

and the last on February 9, 1921; so that the period of shedding pollen, while not continuous, extended over about two months, a very favorable feature in securing hybrids.

The sporangia are in two rather widely separated groups, with 10–16 sporangia in a group. Nearly all the sporangia are in pairs,

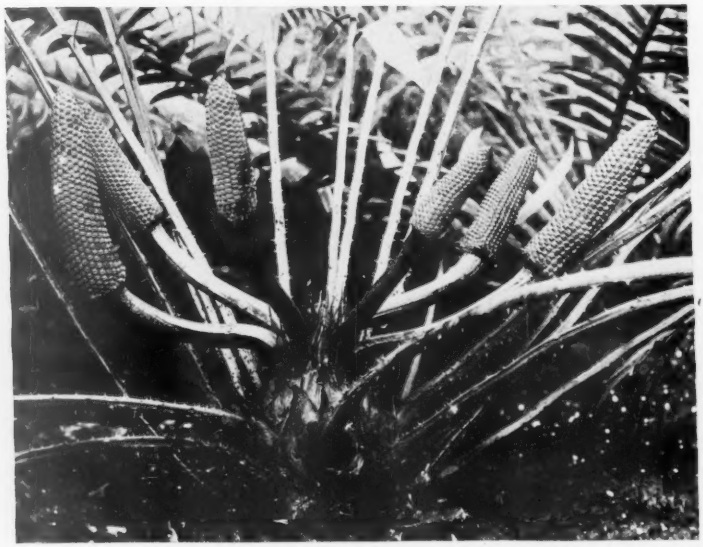

FIG. 1.—*Zamia monticola*, cones of 1920: cone at left reached length of 16 cm. when pollen was shed; leaves in background belong to *Encephalartos;* negative taken in University of Chicago greenhouse by P. J. SEDGWICK.

so that there are only two sporangia in a sorus. Some of the sporangia are single, and, in a few cases, sori of three sporangia were noticed. Nearly all of the sporangia are in rows, with the dehiscence in the longitudinal axis of the sporophyll. All of these features are shown in fig. 2.

Ever since the plant began to cone, new leaves have been produced with each set of cones. In 1919 the crown consisted of six leaves, which reached a length of 1.7 m. The crown of 1923 contained eighteen leaves, and the latest crown, that of 1925, consists of twenty-one leaves, the largest of which are 1.7 m. in length.

The lower part of the petiole, below the lowest leaflets, is very spiny, but there are very few spines in the leafy portion. The number of leaflets varies from thirty to thirty-four, the upper twenty of which are approximately in pairs; three or four at the base are so

FIG. 2.—*Zamia monticola:* microsporophylls in various positions, all showing characteristic sori of two sporangia; middle sporophyll in top row shows one sorus with three sporangia; negative by C. Y. CHANG.

scattered that there is no indication of pairing; and the rest are intermediate between the alternate and the paired condition. The larger leaflets are 24–26 cm. in length, and 3.5–4 cm. in width. The lowest leaflet is sometimes as small as 7 cm. in length and 1.2 cm. in width, but there is no tendency to extreme reduction.

The number of veins, 1 cm. from the base of the leaflet, is about fifteen; and the widest part, about thirty. As the leaflet tapers from

the widest part to the tip, the veins are lost in the margin, usually with no tendency to produce serration, but sometimes producing a distinct serration near the tip (fig. 3). Most of the leaflets are as

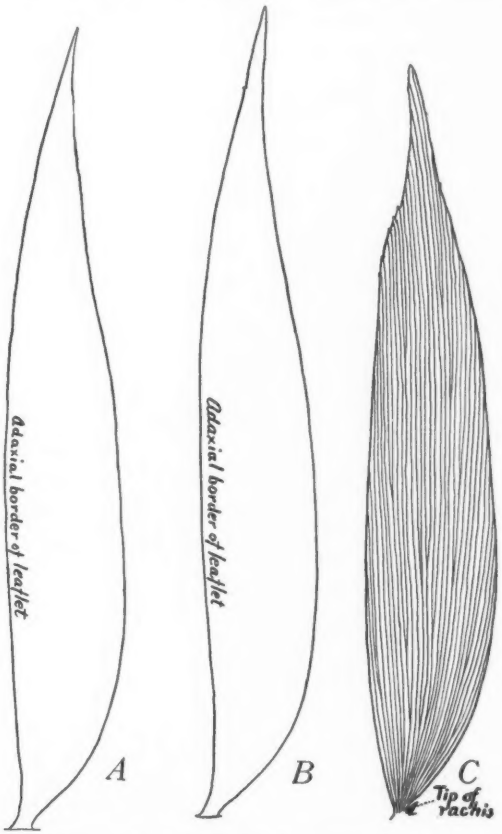

FIG. 3.—*Zamia monticola*, three leaflets: *A* (entire) and *B* (almost entire), below middle of leafy portion; *C*, one of terminal pair, showing almost maximum serration and also venation; one-half natural size.

entire as those of *Ceratozamia*, a feature which might be a partial excuse for not noticing earlier that the plant is a *Zamia*. The leaflets on the lower half of the leafy region are practically free from serration, and so far as their contour is concerned, many of them might

be mistaken for *Ceratozamia*, also accounting for not noticing a plant of *Zamia* in a patch of *Ceratozamia;* but in practically every leaf some of the upper leaflets show serration (fig. 3).

With so many large leaves in a crown, and crowns produced in such rapid succession, it is natural that the stem should develop rather rapidly. At present, the trunk above ground is 21 cm. high and 14 cm. in diameter. The armor is well developed on the part above ground, with no indication of being reduced, as in the tuberous species of the genus.

The characters described are summarized in the following diagnosis:

Zamia monticola, sp. nov.—Male cones oblong-ovoid, 12–16 cm. long; peduncles 10–17 cm. long, much expanded at base of cone and between horizontal and erect position. Microsporophylls hexagonal in surface view, sporangia in two widely separated groups with 10–16 sporangia in a group, sporangia mostly in pairs. Female cones not known. Leaves numerous, 20 or more in a crown, 1.3–1.7 m. in length, lower part of petiole spiny, 30–34 leaflets with upper 20 approximately in pairs and the rest scattered; larger leaflets 24–26 cm. long and 3.5–4 cm. wide, mostly entire but some serrate near the tip; about 30 veins in the widest portion. Stem arborescent.—On mountain side near Jalapa, Mexico, opposite the extinct crater of Naolinco.

Zamia sylvatica

This species was secured in September 1910 from the dense forest across the Papaloapan River south of Tuxtepec. The distance was probably not more than five miles beyond the river. A few miles farther on, *Dioon spinulosum* occurs in great abundance. It also has rather large leaves for a *Zamia*, those of this specimen reaching 1 m. in length, but produced sparingly, with only 2–4 in a crown, and coming at long intervals. At present the plant has only one leaf (fig. 4). The petioles are quite free from spines.

The next spring after the plant was set out, it produced two leaves; two more appeared in April 1915, and a crown of four leaves came in 1920. All of the leaves are about 1 m. in length. The average number of leaflets is about 34, and nearly all of them are more or less paired (fig. 4). The largest leaflets are 32–34 cm. long, and

2.3–2.6 cm. wide. The number of veins at the widest part of the leaflets varies from 40 to 46. The leaflet is entire below the middle, but is finely and sharply serrate near the tip (fig. 5). In the spring of 1921, four leaves and also a female cone appeared, which, without pollination, reached a length of 10 cm., with a peduncle 11 cm. long (fig. 6). Before taking this negative, some of the top soil was removed, and it became evident that the plant was a branching specimen, with two leaves on one branch and two leaves and the cone on the other.

FIG. 4.—*Zamia sylvatica:* plant with one leaf, 1 m. in length, in University of Chicago greenhouse; negative by C. Y. CHANG.

The sporophylls, while spiral, show a longitudinal arrangement to an unusual degree. In surface view, the transverse ridge is a little above the middle of the sporophyll, and extends entirely across. From the ridge, the sporophyll slopes toward the top and the bottom, but about 1 mm. from the bottom the angle changes, and the final millimeter is parallel with the axis of the cone (fig. 6). Even in the illustration, which is about two-thirds natural size, the parallel portion can be distinguished. If pollination and fertilization had taken place, the cone would undoubtedly have been larger. All the ovules were abortive. Male cones are unknown. The larger branch of the stem is 8 cm. in diameter, and the smaller, which bears the cone, measures 5 cm.

The following diagnosis, while lacking the male cone and the seed, will serve to identify the species:

Zamia sylvatica, sp. nov.—Female cone cylindrical, 10 cm. or more in length, 5.5 cm. in diameter. Scales in surface view hexagonal

FIG. 5.—*Zamia sylvatica:* upper part of leaf, showing fine but sharp serration near tips of leaflets; negative by C. Y. CHANG.

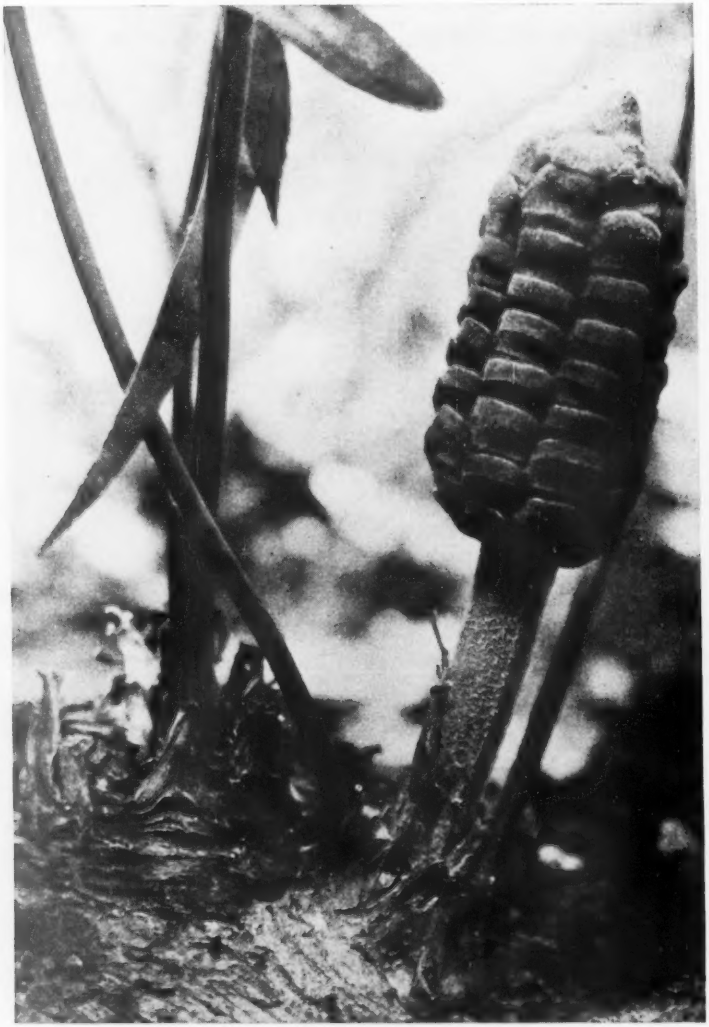

FIG. 6.—*Zamia sylvatica:* upper portion of branched plant; one branch bearing two leaves, and the other two leaves and a female cone; cone 10 cm. in length, with peduncle nearly as long; negative by P. J. SEDGWICK.

with transverse ridge extending entirely across, and the lower millimeter of the lower part of the sporophyll parallel with the axis of the cone. Male cone unknown. Leaves about 1 m. in length, with 2–4 in a crown. Petiole smooth. Leaflets about 32, approximately in pairs, the longest leaflets 32–34 cm. in length and 2.3–2.5 cm. in width; number of veins in the widest part of the leaflet 40–46. Leaflets entire below the middle, finely and sharply serrate toward the tip. Stem subterranean.—In the forest about five miles south of the Papaloapan River at Tuxtepec, Mexico.

It is very probable that a careful examination of the regions cited for these two species of *Zamia* would yield other cycads, besides undescribed plants in other groups.

University of Chicago

BRIEFER ARTICLES

THE AUXIMONE QUESTION

It is more than ten years now since BOTTOMLEY[1] conceived that plants are unable to continue healthy and normal growth in purely inorganic cultures, but require certain accessory organic substances, for which he proposed the name *auximones*.[2] For some years he and his pupils pursued this principle experimentally, and established it to their own satisfaction for cultures of *Lemna*,[3] only MENDIOLA[4] dissenting openly from their view.

In the summer of 1922 the writer undertook to test the correctness of this theory, and carried on certain experiments. These were never reported, because they were felt to be incomplete, and it was hoped to complete them. In the past year, however, two papers have been published dealing with this question, one of them giving some of the data for lack of which the writer's results were withheld from publication. Since interest is again aroused at this time, and since the writer's experiments seem to make a timely addition to our present published knowledge, it is deemed wise to offer now the results of this former work, together with a critical review of the whole situation to date.

BOTTOMLEY used pond water and the standard nutrient solutions of KNOP and DETMER as culture media, and was unable to maintain the health of *Lemna* and other water plants in these two artificial media. The fact that SHIVE[5] had secured notably better results with wheat grown in a nutrient solution of his own devising than in Knop's solution, suggested that BOTTOMLEY was not supplying a properly balanced nutrient solution, the lack of inorganic balance being made good by the organic additions termed "bacterized peat extracts." Accordingly, a series of cultures

[1] BOTTOMLEY, W. B., Some accessory factors in plant growth and nutrition. Proc. Roy. Soc. Lond. B **88**:237–247. 1914.

[2] ———, A bacterial test for plant food accessories (auximones). Proc. Roy. Soc. Lond. B **89**:102–108. 1915.

[3] ———, The effect of auximones on the growth of *Lemna minor* in nutrient solutions. Proc. Roy. Soc. Lond. B **89**:483–506. 1917 (SAEGER gives very complete citations).

[4] MENDIOLA, N. B., Variation and selection within clonal lines of *Lemna minor*. Genetics **4**:151–182. 1919.

[5] SHIVE, J. W., A 3-salt nutrient solution for plants. Amer. Jour. Bot. **2**:157–160. 1915.

of *L. minor* was tried with Series 1 of the 3-salt nutrient solutions for water cultures advocated by the National Research Council,[6] using Shive's Best for Wheat as control.[7] Of the Series 1 solutions, no. 7 gave the best growth, but the control far surpassed it. In neither case was there any sign of loss of vigor and healthiness during the five weeks'

TABLE I

Inorganic media, 36 days		Inorganic+organic media, 20 days		
Culture no.	Percentage gain	Organic addition	ppm	Percentage gain
1.....	1500	Arabinose	200	550
2.....	1350	Alanine	65	170
3.....	1200	Asparagine	200	200
4.....	1100	Caffeine	65	0
5.....	1150	Dextrose	200	600
6.....	900	Diphenylamine	65	300
7.....	1750	Galactose	200	600
8.....	1000	Phenylhydrazine	65	0
9.....	1100	Piperine	65	750
10.....	1150	Pyridine	200	460
11.....	1400	Urea	200	160
12.....	1450	Urea	150	220
13.....	1400	Urea	100	280
14.....	1150	Urea	50	730
15.....	1000	Control	.....	900
16.....	1900	Control	.....	940
17.....	1150	.....	.....	.....
18.....	1250	.....	.....	.....
19.....	1500	.....	.....	.....
20.....	1400	.....	.....	.....
21.....	1200	.....	.....	.....
Control....	2750 (about 950 in 20 days)			

duration of the experiment, whereas Bottomley reported unhealthiness from the start with Detmer's solution. The left side of table I presents in full the results of this experiment, the increase in number being corrected for size, so that the figures represent practically increase in area. Culture 16 shows a higher figure than no. 7, but the plants in it were in not nearly such healthy condition nor so large. Thus it seemed almost certain that the previous failures to maintain normal growth in purely inorganic

[6] Livingston, B. E. (ed.), A plan for cooperative research on the salt requirements of representative agricultural plants. 2d. ed. Baltimore. 1919.

[7] It has been found that owing to an error in the directions of the National Research Council bulletin, the control solution used in the writer's experiments was three and one-half times as dilute as Shive's Best, which was supposed to be the control.

media were due to a lack of proper physiological balance, and not to specific need of hypothetical accessory organic factors.

The next step was to determine what would be the effect of the addition of small quantities of organic matter to a physiologically balanced inorganic nutrient solution. Instead of the unknown factors added in the bacterized peat extract, various known compounds were used in low concentrations. These compounds, their concentration (in parts per million), and the results of their addition to the control used in the inorganic experiments are shown on the right side of table I. In no case was growth so good in the presence of these organic additions as in their absence, although the small quantities added can hardly have exercised any specific toxicity, in the case of the sugars at least.

Turning now to a consideration of the work of previous writers, we find that MENDIOLA noted that a modified Pfeffer's solution gave good growth of *Lemna*, but the observation was rather an incidental one only. CLARK and ROLLER[8] obtained even better growth in certain inorganic media than did BOTTOMLEY with Detmer's solution plus bacterized peat extract, the rate of reproduction being maintained at a high value in their experiments, while in his it was initially high but soon fell off. They employed 125 cultures, using this number of various combinations and concentrations of the salts of Series 3 of the National Research Council's bulletin. Unfortunately they state only that they found several combinations which were satisfactory, and do not state the actual constitution of these solutions, so that their work cannot readily be made the basis of further investigation.[9] They seem to show very conclusively, however, that organic matter is unnecessary in nutrient solutions if a proper physiological balance of inorganic salts is maintained.

Both these workers and the writer failed to employ the media used by BOTTOMLEY, and it was for this reason that the writer felt his own work to be inconclusive and incomplete. Very recently, however, SAEGER[10] has published an account of experiments in which the media used by BOTTOMLEY were compared with others, in this case simply various dilutions

[8] CLARK, N. A., and ROLLER, E. M., "Auximones" and the growth of the green plant. Soil Sci. **17**:193–198. 1924.

[9] Since the preceding was written, CLARK has published a paper (CLARK, N. A., The rate of reproduction of *Lemna major* as a function of intensity and duration of light. Jour. Phys. Chem. **29**:935–941. 1925) in which he gives the exact composition of one of the solutions found satisfactory.

[10] SAEGER, A., The growth of duckweeds in mineral nutrient solutions with and without organic extracts. Jour. Gen. Physiol. **7**:517–526. 1925. (This article has a very complete bibliography appended, especially of BOTTOMLEY's work.)

of these same. With ten times dilutions of both Detmer's and Knop's solutions, excellent growth was obtained of a species nearly related to that used by BOTTOMLEY, while his results were confirmed by the poor growth and sickliness of the plants grown in the normal strength of these solutions. Here again there is demonstrated the lack of need of organic substances in media.

Using the Knop/10 solution, SAEGER examined the effect of small quantities of autolyzed yeast and of peat extract, and noted in both cases considerable increase of growth, although the same was true if the natural pond water was substituted for the artificial solution and these organic substances of unknown nature were added thereto. No explanation, of course, is possible for the catalytic effect of these substances, and their unknown nature only aggravates the difficulties already presented. It is probable that there are other inorganic media which would give even better growth than the diluted Knop's solution, but since SAEGER used a different plant in his cultures it is not possible to compare his results with any others.

In view of the results of these four independent sets of experiments, all of which refute BOTTOMLEY'S theory completely, and point to lack of physiological balance as the difficulty which led to its proposition originally, it seems that we may well drop from the literature the term *auximones*. Whether in a physiologically balanced inorganic nutrient medium it is possible to stimulate growth still further by the addition of organic substances is beside the question, since auximones were hypothecated as absolutely indispensable for the continued healthy growth of a plant, and not simply as capable of increasing growth. The work of CLARK and ROLLER and of the writer would seem to indicate that such additions will not increase the rate of growth, and while SAEGER has obtained some increase, it must be remembered that he did not assure himself of having the best inorganic medium possible initially, and that his unknown extracts also contained inorganic as well as organic matter. It is, of course, impossible to compare directly the results of two different experimenters quantitatively, because of the great differences in the environmental conditions and in the hereditary factors involved in the two cases. We await with interest further experimentation in which these various difficulties shall be eliminated; but whatever the results, auximones as conceived by their namer do not exist.—H. S. WOLFE, *Department of Botany, University of Chicago.*

CURRENT LITERATURE

BOOK REVIEWS

Photosynthesis

Many investigators have attempted to obtain knowledge of how the plant utilizes radiant energy in the manufacture of organic compounds. A useful summary of the literature of this problem has been prepared by STILES,[1] who has not neglected any important section of the world's work dealing with this subject. The book is in no sense a mere revision of the earlier review by JØRGENSEN and STILES. It presents a more general view of the developments since the days of PRIESTLEY and INGEN-HOUSZ, but preserves the admirable critical spirit in handling all the recent contributions to our knowledge of this difficult problem.

The introductory chapter gives a rather brief glimpse of the history since the phlogiston idea of carbon was abandoned. This chapter could have been made more extensive with profit to the students of today. Following the introduction, there is a short discussion of the system involved in the food manufacturing cells and tissues. The pigments, chlorophylls, and carotinoids are described, and methods of demonstrating photosynthesis are presented. Beginning with chapter v, the author takes up the main questions, methods of measurement, intake of CO_2, influence of internal and external conditions on the process, the products of synthesis, utilization of energy, the mechanism as a chemical process, and the relation of photosynthesis to other processes of life. The concluding remarks summarize the progress made, and point the way to larger advancement.

With reference to the work on the mechanism of photosynthesis, the present situation is summarized by quoting SACHS' statement: "Whether it is right to claim . . . . formic acid or some other member of the formyl group as the first product of assimilation on account of its simple constitution, I hold as at least very questionable; and it has hitherto been proved by nothing." This quotation, from the nineteenth chapter of *Lectures on the physiology of plants* (1882), may be as truly applied to the more recent hypotheses suggested to account for the conversion of carbonic acid into sugar in green plants.

The book presents a bibliography of nearly 900 citations, which is a great convenience to the student who desires to consult the original sources. It is written in simple, readable style, and avoids the mass of details which would only obscure the picture.—C. A. SHULL.

[1] STILES, WALTER, Photosynthesis. 8 vo. pp. viii+268. *figs.* 15. London: Longmans. 1925.

Textbook of plant physiology

A concise textbook of plant physiology has been written by LEPESCHKIN,[2] who treats the subject from the physico-chemical point of view. The introduction discusses the peculiarities of living matter and physiological phenomena, colloidal behavior of living protoplasm, external and internal conditions of life, irritability, mechanism, and the classification of physiological phenomena in plants.

The discussion then proceeds in three sections: metabolism, growth, and movement. Metabolism is considered in five chapters: first the general physico-chemical basis of metabolism, then chapters on water in the plant, minerals, organic compounds, and respiration. Two chapters on growth present the general physico-chemical basis of cell growth and reproduction, and a descriptive and explanatory treatment of growth phenomena, as influenced by temperature, light, humidity, stimulation, nutrients, and toxins. This chapter includes some discussion of correlation and transplantation of tissues. The section on movements is similarly divided into a general discussion of the physico-chemical basis for the phenomena of movement, and a descriptive and explanatory discussion.

No attempt has been made to summarize the entire literature of plant physiology, but to present a concise, well organized statement, citing only the most important literature, the outstanding contributions. The author succeeds remarkably well, in a book of such small compass. Some may object to the small space given to the growth phenomena, which occupy only 68 pages, and to the anticlimax provided by the section on movements. Notwithstanding these matters of emphasis and organization, the author has given us a useful brief summary of this fundamentally important science.—C. A. SHULL.

Cyclopedia of horticulture

The great success of BAILEY'S *Cyclopedia of horticulture* is well attested by its numerous reprints and revisions. There has recently appeared a reprint of the entire Cyclopedia, in three volumes instead of the customary six volumes of previous editions.[3] The bulk of the volumes has been very much reduced by the use of thinner paper, which, however, is strong and easily turned. This edition of the Cyclopedia is very handsome and satisfactory, and is much the most attractive and easily usable of the long series which started a quarter of a century ago.—H. C. COWLES.

NOTES FOR STUDENTS

Carbohydrates in nitrogen metabolism.—Etiolated seedlings differ in their power to utilize ammonia as a source of nitrogen. High carbohydrate seedlings, barley for instance, easily form asparagin from ammonium salts, while some of

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[2] LEPESCHKIN, W., Lehrbuch der Pflanzenphysiologie auf physikalisch-chemischer Grundlage. 8vo. pp. vi+297. *figs. 141.* Berlin: Springer. 1925.

[3] BAILEY, L. H., The standard cyclopedia of horticulture. Vols. I–III. pp. xxiv+639. *pls. 120 (24 colored). figs. 4000.* New York: Macmillan Co. 1925.

the legumes cannot use ammonia compounds unless $CaCO_3$ is present to keep down acidity. The yellow lupine among legumes has been found by PRIANISCHNIKOW[4] to be exceptional, in that even when the carbonate is present it cannot use ammonia as a source of nitrogen. Instead of building up proteins, it stores the ammonia as such in its tissue, and such proteins and amides as have already been formed are converted into ammonia, so that the stored ammonia represents more than that absorbed. The plants exhibit ammonia toxicity under these circumstances. He finds that this peculiar behavior is not hereditary, but depends upon internal conditions of nutrition. If the etiolated seedlings are fed on soluble carbohydrates, or if active green seedlings are used in light, so as to convert them from low to high carbohydrate seedlings, in either case the lupines can use the ammonia to build asparagin. There is no accumulation of ammonia in the tissue. In other words, the lupines behave like barley when like barley they have a high carbohydrate supply. Again, if the barley seedlings are starved by exhaustion of the reserves before use, or if the endosperm is cut away, they lose their power to form asparagin from an ammonia supply, and behave like lupines.

PRIANISCHNIKOW considers that the nitrogen metabolism is a reversible chemical reaction, running from proteins and amino acids by oxidation and secondary synthesis to amides, and from these by further oxidation to ammonia, especially in the absence of sufficient carbohydrate. When sufficient carbohydrates are present the process is reversed, and ammonia can be utilized to form amides, which then build up into amino acids and proteins. In such a system, changing the quantity of carbohydrate reverses the whole process, and the amount present will determine whether the plant can build proteins, or whether it will store nitrogen as ammonia. Ammonia is considered the first step in synthesis, the last in the disintegration of the proteins. The same principles apply when nitrates are the nitrogen source, only that the nitrogen must first be reduced to the condition of ammonia. The work throws interesting light on the carbohydrate-nitrogen ratio which has assumed importance in the literature in recent years.—C. A. SHULL.

Japanese vegetation.—The Island of Yezo lies to the north of Honshu, the main island of Japan, and between 41° 24′ and 45° 31′ north latitude. It is therefore in the same latitude as northern United States, and a recent description of its vegetation[5] serves to emphasize the similarity of the plant communities of the two regions. The forests are said still to cover more than half the whole area, and are made up of broad leaved deciduous, conifer, and mixed types, the conifers being more plentiful toward the north.

The largest trees are *Populus Maximowiczii*, *Cercidophyllum japonicum*, and

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[4] PRIANISCHNIKOW, D., Über den Aufbau und Abbau des Asparagins in den Pflanzen. Ber. Deutsch. Bot. Gesells. 40: 242–248. 1922.

[5] KUDO, YUSHUN, The vegetation of Yezo. Jap. Jour. Bot. 2: 209–292. 1925.

Quercus grosseserrata, while among the most widely distributed species are *Salix jessoensis*, *Ulmus japonica*, *U. laciniata*, *Betula japonica*, *B. Ermani*, *Morus bombycis*, *Alnus hirsuta*, *Prunus serrulata*, *Tilia japonica*, *T. Maximowicziana*, *Acer pictum*, and *Fraxinus pubinervis*. In addition there is a group of genera such as *Magnolia*, *Styrax*, *Picrasma*, *Ostrya*, *Carpinus*, and *Pterocarya*, which occur but are more characteristic of the southern main island. Among the conifers, some of the more abundant are *Pinus pentaphylla*, *Abies sachalinensis*, *A. mayriana*, *Picea jezoensis*, *P. Glehni*, *Larix dahurica* var. *kamtschatica*, and *Thujopsis dolabrata*. Notes are given on the habits of these and other tree and shrub species. The number of woody plants is 256, including 24 woody climbers, and the entire vascular flora includes 1629 species. The island is divided by a central depression into an eastern and a western region. The latter is much the smaller, being only one-sixth of the whole, and presents a hilly and mountainous surface, but with no peaks above 5000 feet. The eastern division on the other hand has broad plains and higher mountains, culminating in Mt. Taisetsu, 7500 feet. With an unusual precipitation ranging from 80 to 116 cm., the six botanical districts exhibit plant associations and formations very similar to those of eastern North America. These districts are: (1) southwestern Yezo, dominated by birch forests and showing a close affinity to northern Honshu; (2) southeastern Yezo, showing southern affinities but dominated by fir and spruce forests; (3) central Yezo, with an abundance of bogs and alpine meadows, but with deciduous forests on the plains becoming mixed with conifers on the mountain slopes; (4) eastern Yezo, with many swamps in the western portion of the district, including extensive associations of *Phragmites communis* and forests of *Abies sachalinensis* and *Picea Glehni* in the west; (5) Province of Kitami along the northeast coast, similar to District 3 but with more northern affinities; and (6) southern Kurile Islands, similar to District 4 but with decidedly fewer species.

The floristic affinity of Japan and New England, so excellently described by GRAY, is made still more evident by the analysis here given by KUDO. His list of species gives not only their occurrence in Yezo and elsewhere in Japan, but also in many other countries. Thus the relationship of the flora of Yezo and other regions is shown by the fact that 79 per cent of its species are common to Honshu, and over 38 per cent are also common to Korea, Kyushu, Shikoku, China, Manchuria, and Saghalien; while Siberia, Europe, and North America each have more than 26 per cent of the species now recorded for the island.

An examination of orders and genera shows that the Pteridophytes include 30 genera with 98 species, the Taxaceae 2 genera of 1 species each, the Pinaceae 6 genera with 11 species, and the angiosperms 122 families, 559 genera, and 1518 species. Some of the larger families, in the order of size and number of species, are Cyperaceae 142, Gramineae 117, Compositae 116, Rosaceae 73, Liliaceae 64, Orchidaceae 61, Ranunculaceae 55, Cruciferae 44, Umbelliferae 39, Labiatae 38, Ericaceae 38, Leguminosae 38, Caryophyllaceae 36, Polygonaceae 36, Scrophulariaceae 35, Saxifragaceae 35, and Violaceae 32.—GEO. D. FULLER.

A genetic, ecologic, and taxonomic study of Hemizonia.—A recent paper by BABCOCK and HALL[6] marks a definitely forward movement in taxonomy, because it approaches the question of species by a combination of methods of different fields. It has long been the opinion of the reviewer that the concept of species is not a purely taxonomic matter, but that other fields of botany also must contribute to its understanding. In 1836 DE CANDOLLE first described *Hemizonia congesta* as the first species of a new genus which he established. At the same time he recognized and described a second species. Prior to 1884 four other species were recognized by various taxonomists. In 1884 the total number of species was reduced to three by ASA GRAY. Since that time two more have been described. By the combination study of the authors, the species now have been reduced to one, namely, *Hemizonia congesta*. The other so-called species are found to be only subspecies. This conclusion is not due to a hasty study but to one that has covered years. HALL began his taxonomic and ecologic study of *Hemizonia* in 1912, and BABCOCK began his genetic study in 1915. Nor does the conclusion mean a shifting of species concepts. It means rather access to a greater abundance of material, accompanied by adequate field study and experimentation. The six subspecies have innumerable forms, some of which are genetic and can be maintained by selection. The subspecies are roughly correlated with geographic distribution. There are no differences in the number or individuality of the chromosomes. Artificial hybrids are easily produced and sometimes they develop supposed natural hybrids. Since the inheritance is Mendelian, the evolution of the subspecies is thought to be by mutation. Natural selection of favorable mutations accounts for the existence of five of the subspecies, and the geographic distribution of three subspecies indicates the importance of isolation in establishing them. It is to be hoped that the future will see many more joint investigations of this sort. Whether or not such studies will radically decrease the number of so-called species is a minor matter, although most botanists regret the indefinite multiplication of "species." The real value of contributions of this sort is to put taxonomy on an absolutely sound and scientific basis, and in line with modern experimental science.—H. C. COWLES.

An ecological study in the Himalayas.—An interesting paper has just appeared from the hands of DUDGEON and KENOYER[7] on the ecology of Tehri Garhwal, which is a state of the western Himalayas with an altitudinal variation of over 20,000 feet. Three great zones of vegetation are recognized, corresponding to three climatic zones, essentially as recognized in SCHIMPER'S *Plant geography*. The lowest of these zones is occupied by the tropical monsoon forest which

[6] BABCOCK, E. B., and HALL, H. M., *Hemizonia congesta*, a genetic, ecologic, and taxonomic study of the hay-field tarweeds. Univ. Calif. Publ. Botany **13**:15–100. 1924.

[7] DUDGEON, W. S., and KENOYER, L. A., The ecology of Tehri Garhwal: A contribution to the ecology of the western Himalayas. Jour. Ind. Bot. Soc. **4**:233–285. 1925.

extends to an altitude of 5000 feet. Above this are sclerophyll forests with broad leaved trees, with an altitude range from 5000 to 11,000 or 12,000 feet, depending upon circumstances. Finally, there are alpine formations in the higher stretches of the mountains. The monsoon forests, unlike some others of India, are dominated by species of *Bauhinia*, and the sclerophyll forests are dominated by various species of *Quercus*. Interesting forests of *Pinus longifolia* and *Cedrus Deodara* are also described. These are not regarded as parts of the climatic climax forest but rather as edaphic climaxes. The pine edaphic climax lies partly in the monsoon forest region and partly in the sclerophyll forest, whereas the deodar edaphic climax lies entirely within the latter region. In the timber line areas occur *Betula utilis* and *Abies Webbiana*.

The latter portion of the paper is taken up with a discussion of the human factors and their influence on the native vegetation. On account of the very dense population, the pressure of human factors is enormous. In the lower altitudes there is found a relatively scant development of the natural formations. The climax vegetation is almost destroyed by man, following which still more disastrous effects are brought about by erosion. Especially serious is the complete destruction of the forest over considerable areas through logging operations, which are followed by severe erosion and the development to some extent of xerophytic pioneers. There is much burning of the dead vegetation during the dry spring, which serves to prevent forest regeneration, especially in the pine forests. These destructive human activities have been taking place for a long time, and seem to be getting worse rather than better.—H. C. COWLES.

Absorption of cations by protoplasm.—SCARTH[8] has studied the penetration of cations into living protoplasm. A physiological reaction was used as a test of penetrability. This consisted of the contraction along their long axes of the chloroplasts and cytoplasmic strands of *Spirogyra*. Especially in the case of many bivalent and trivalent cations, there was observed a retarding of penetration as time proceeded, this flattening out of the curve being more marked in the case of the higher concentrations. Self-antagonism of ions is given as one probable factor in causing this decrease in the rate of penetration. The mechanism of self-antagonism is regarded as the same as antagonism between ions, the seat of the reaction appearing to be the lipoid exterior of the protoplasm. So two reactions of the cell control entrance of the ions, one tending to bring about active absorption of the ion, the other tending to exclude the ion. The sensitiveness of both of these reactions increases with the valency of the ion. As the atomic weight of the ion increases, the factor bringing about active absorption also increases. In the case of the heavy metals, this factor also increases inversely with the electrolytic solution pressure of the ions. This is not true of the other ions. The order of the ions as regards initial penetrability is the same in

[8] SCARTH, C. W., The penetration of cations into living protoplasm. Amer. Jour. Bot. 12:133–148. 1925.

general as their order arranged on the basis of their physiological activity. The author recognizes that it is impossible at present to explain the physiological series of ions by any single physico-chemical law, and so describes the absorption of ions by the protoplasm and their passage through it as a "vital function." He realizes, of course, that this does not explain the process, and states that adsorption seems to be one factor involved, the absorption curve resembling an adsorption curve. That absorption is not a simple diffusion process, is shown by the fact that the rate is not proportional to the diffusion gradient. Problems connected with the absorption of ions by protoplasm are among the hardest and most important confronting plant physiology, and deserve much more work in the effort to solve them.—S. V. EATON.

Relation of light to growth.—ADAMS[9] reports the results of experiments on the relation of light to growth. Under continuous electrical illumination the castor bean was the only species worked with which completed its entire life cycle from seed to seed, although several other species flowered. Some species made better growth in daylight, others in daylight supplemented with electrical illumination. Experiments on the duration and intensity of light showed that two hours' exposure to light at midday gave as good results as three hours' exposure during either the morning or the afternoon. Results of experiments are reported also on the interrelation between temperature and duration of exposure to light, and on the effect on growth of interposing a screen of colorless glass.

Using the tomato, pepper, and nasturtium, DEATS[10] obtained results in agreement with those of GARNER and ALLARD. The amount and rate of growth in each species were proportional to the length of the daily exposure to light, but the requirements for flower formation differed in the different species. The tomato behaved as a long-day plant, the pepper as a short-day plant. Preliminary work with the nasturtium indicated that it too is a short-day plant. Anatomical studies were made on the effect of the length of illumination on bast and xylem development, on cork development, on size and thickness of leaves, and other features. Starch was the only compound determined, the amount of this being determined in a qualitative way by microchemical means. It was found to be greater in the long-day plants. The short-day plants were small, pale, and yellowish, and contained little starch. They had been grown with a good supply of nitrates. Reference is made to unpublished work of ELTINGE, who, working with the same species but varying the nitrate supply, much as did KRAUS and KRAYBILL, found that the plants grown without nitrogen were also undeveloped and of a poor color. They contained much starch

[9] ADAMS, J., Some further experiments on the relation of light to growth. Amer. Jour. Bot. 12:398–412. 1925.

[10] DEATS, M. E., The effect on plants of the increase and decrease of the period of illumination over that of the normal day period. Amer. Jour. Bot. 12:384–394. 1925.

however. The author points out that the two sets of experiments illustrate two of the conditions mentioned by KRAUS and KRAYBILL as causing poor vegetation and non-fruitfulness, namely, high nitrate supply and low carbohydrates, or low nitrate supply and conditions of abundant carbohydrate production. The author recognizes that fuller chemical analyses are needed in experiments of this kind. The general conclusion of the work is that different lengths of day influence the development of plants by changing the carbohydrate-nitrogen ratio.—S. V. EATON.

Sex characters.—Two papers have recently appeared from the California Agricultural Experiment Station dealing with problems of sex. ROBBINS and JONES[11] have discovered some interesting secondary sex characters in *Asparagus*. Staminate plants express their sex earlier in life than do pistillate plants. The average height of the first flower-bearing shoot of staminate plants is not so great as that of the pistillate plants. There is also a marked difference in the green weight of the tops, that of the staminate plants being considerably greater than that of the pistillate. Other differences were noted also as to yield and weight of spears, the staminate plant exceeding the pistillate in both particulars.

ROSA[12] has investigated spinach, which is a dioecious, wind-pollinated plant. He distinguishes the sex arrangements under four categories: "extreme males," bearing only staminate flowers with reduced or even suppressed leaves on the flower branches; "vegetative males," bearing only staminate flowers, but with well developed leaves on the flower branches; "monoecious" plants, bearing staminate and pistillate flowers in variable ratios in the same cluster, and with fully developed leaves on the flower branches; and "female" plants, bearing only pistillate flowers and fully developed leaves. In consequence, he calls spinach a "tetra-morphic" plant, although there are intergrading forms. He finds that environment has no effect in determining these types, and therefore the differences seem to be due to genetic factors. Tested in several ways, as influence of rich and poor soils, of shade and light, of early and late planting, of mutilation, no response in sex expression was discovered.—J. M. C.

Evolution of Vernonia.—GLEASON[13] has made a comparative study of the North American species of *Vernonia* in reference to their relationships and distribution. The so-called sections are remarkably unequal in their representation, three of them including only a single species each, and one of them represented by 120 species. He has discovered that characters which are held to represent primitive conditions in one group may indicate advanced evolution

[11] ROBBINS, W. W., and JONES, H. A., Secondary sex characters in *Asparagus officinalis* L. Hilgardia 1:183–202. 1925.

[12] ROSA, J. T., Sex expression in Spinach. Hilgardia 1:259–274. 1925.

[13] GLEASON, H. A., Evolution and geographical distribution of the genus *Vernonia* in North America. Amer. Jour. Bot. 10:187–202. *figs.* 3. 1923.

in another, and that such characters have no apparent relation to environment. In every case the groups which have the simplest morphological features occur to the south, while the more complex groups appear progressively farther toward the north. He concludes that evolution and migration have proceeded together.—J. M. C.

Physiological studies of Phytophthora.—LEONIAN.[14] has investigated the genus *Phytophthora* with the purpose of securing a less difficult and more reliable taxonomy. The specific distinctions have been so limited and variable as to make the segregation of species very unreliable. Cultures were made of 53 strains, representing most of the described species, and responses to various media noted. As a result, these responses enabled the investigator to recognize specific reactions, and to construct a physiological key. *Pythiacystis citrophthora* has been transferred to *Phytophthora;* a number of so-called species have been merged in *P. omnivora;* and *P. pini* has been described as a new species which occurs on the roots of *Pinus resinosa* in Minnesota. Another interesting result of this investigation was the study of saltation phenomena, the conclusions being that no new species or varieties have been produced in this way.—J. M. C.

Embryo sac of Moringa.—RUTGERS[15] has discovered an interesting situation in the development of the embryo sac of *Moringa*, a tropical genus indigenous in the Indies, but long cultivated also in the African and American tropics. The chalazal megaspore functions, and by two successive divisions two nuclei occur at each pole. The third division is restricted to one of the micropylar nuclei, resulting in a 5-nucleate sac. A normal egg apparatus is organized, and the second male cell fuses with the two chalazal nuclei to form the endosperm nucleus. This behavior has been reported heretofore only by TREUB for *Garcinia*.—J. M. C.

Leaf spot of maize.—DRECHSLER.[16] has discovered that a leaf disease of maize occurring in Florida and the Philippines is caused by a new species of *Ophiobolus*, which he describes tentatively as *O. heterostrophus*. A detailed description of its life history is given, and the conclusion is reached that the species of *Helminthosporium* "with straight subcylindrical conidial germinating laterally from end and intermediate segments constitute a natural group distinct from the group of species producing curved elliptical conidia germinating by two polar germ tubes." It seems that this disease has been confused with leaf blight, which also occurs in tropical and subtropical regions.—J. M. C.

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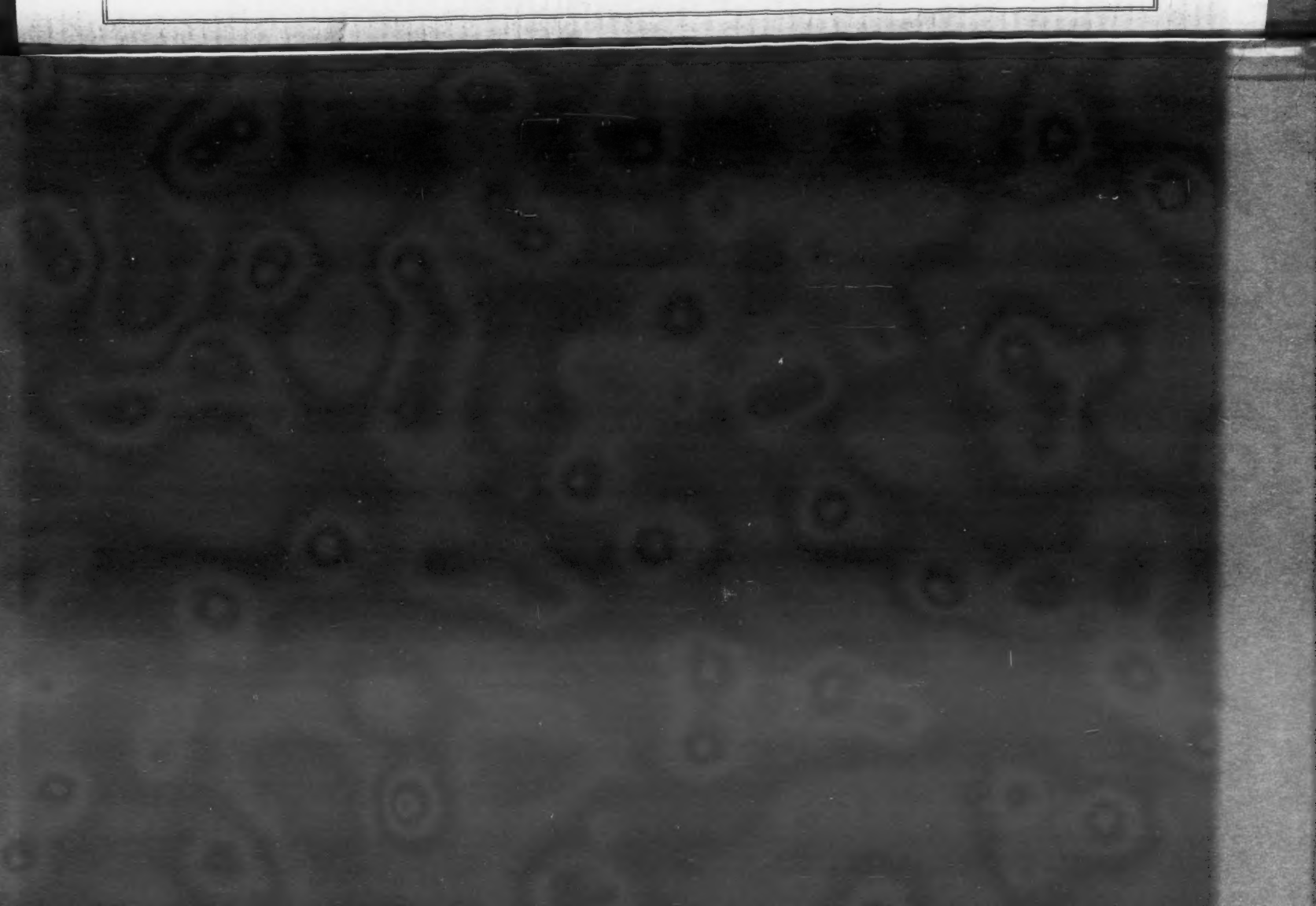
[14] LEONIAN, L. H., Physiological studies on the genus *Phytophthora*. Amer. Jour. Bot. 12:444–498. 1925.

[15] RUTGERS, F. L., Embryo sac and embryo of *Moringa oleifera* Lam. Reliquia Treubianae III. pp. 5. 1923.

[16] DRECHSLER, C., Leaf spot of maize. Jour. Agric. Res. 31:701–726. 1925.



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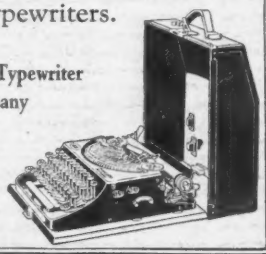

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